# EVALUATION OF HYDROXYL ION DIFFUSION FROM CALCIUM HYDROXIDE MEDICAMENT PLACED IN INTACT AND RESORBED TEETH DURING PRIMARY ENDODONTIC TREATMENT AND RETREATMENT – AN INVITRO STUDY

*Dissertation submitted to*

**THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY**

*In partial fulfillment for the Degree of*

**MASTER OF DENTAL SURGERY**

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## THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY
## CHENNAI

## DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation titled **"EVALUATION OF HYDROXYL ION DIFFUSION FROM CALCIUM HYDROXIDE MEDICAMENT PLACED IN INTACT AND RESORBED TEETH DURING PRIMARY ENDODONTIC TREATMENT AND RETREATMENT – AN INVITRO STUDY"** is a bonafide and genuine research work carried out by me under the guidance of **Dr. P. SHANKAR, M.D.S.,** Professor, Department of Conservative Dentistry and Endodontics, Ragas Dental College and Hospital, Chennai.

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# CERTIFICATE

This is to certify that this dissertation titled **"EVALUATION OF HYDROXYL ION DIFFUSION FROM CALCIUM HYDROXIDE MEDICAMENT PLACED IN INTACT AND RESORBED TEETH DURING PRIMARY ENDODONTIC TREATMENT AND RETREATMENT – AN INVITRO STUDY"** is a bonafide record work done by **Dr. CELENA BENCY. M** under our guidance during her postgraduate study period between 2015 - 2018.

This dissertation is submitted to **THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY**, in partial fulfillment for the degree of **MASTER OF DENTAL SURGERY – CONSERVATIVE DENTISTRY AND ENDODONTICS, BRANCH IV**. It has not been submitted (partial or full) for the award of any other degree or diploma.

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This is to certify that this dissertation work titled **"EVALUATION OF HYDROXYL ION DIFFUSION FROM CALCIUM HYDROXIDE MEDICAMENT PLACED IN INTACT AND RESORBED TEETH DURING PRIMARY ENDODONTIC TREATMENT AND RETREATMENT – AN INVITRO STUDY"** of the candidate **Dr. CELENA BENCY.M** with Registration Number ________________ for the award of **MASTER OF DENTAL SURGERY in BRANCH IV - Conservative Dentistry and Endodontics.** I personally verified the urkund.com website for plagiarism Check. I found that the uploaded dissertation file contains from introduction to conclusion pages and the result shows **1 percentage** of plagiarism.

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# ABSTRACT

**AIM AND OBJECTIVE:**

The aim of this invitro study was (i) to evaluate the hydroxyl ion diffusion from calcium hydroxide intracanal medicament placed in intact and simulated resorbed teeth during primary endodontic treatment and retreatment and (ii) to assess if the hydroxyl ion diffusion from calcium hydroxide medicament during retreatment was dependent on the type of sealer used during primary endodontic treatment done in extracted mandibular premolars using pH meter.

**METHODOLOGY:**

40 single rooted mandibular premolars were divided into 2 groups of 20 teeth each namely Group 1: Intact teeth and Group 2: Simulated resorbed teeth. All teeth were decoronated at CEJ followed by chemomechanical preparation. Intracanal dressing was done with RC Cal and the access cavity was sealed both during primary endodontic treatment and retreatment. The specimens were then individually suspended in 10ml of deionized water. A digital pH meter was used to measure the pH on $3^{rd}$,$7^{th}$,$14^{th}$,$21^{st}$ and $28^{th}$ day. For measuring the pH change after retreatment, the samples in Group 1 and group 2 were further subdivided into 4 groups each based on the sealers used for obturation namely group A (Zinc oxide eugenol sealer), Group B (Sealapex), Group C (MTA fillapex) and group D (BioRoot RCS). Data obtained were compiled and statistically analyzed.

**RESULTS:**

The pH change was most alkaline in both intact and simulated resorbed teeth during primary endodontic treatment and retreatment on the $7^{th}$ day. The dissociation of hydroxyl ions from the intracanal medicament was more in resorbed teeth than in intact teeth during both primary endodontic treatment and retreatment on $3^{rd}$ and $7^{th}$ day, whereas, on the $14^{th}$,$21^{st}$ and $28^{th}$ day it was higher in intact teeth than resorbed teeth. In both intact and resorbed teeth, significantly higher change in pH was seen during primary endodontic treatment than during retreatment at all time intervals. During retreatment following placement of intracanal medicament in both intact and resorbed teeth, highest pH was observed in Group A (Zinc oxide eugenol) followed by Group B (Sealapex), Group C (MTA fillapex) and Group D (BioRoot RCS) during the entire period of study.

**CONCLUSION:**

The present study showed that the change in pH due to dissociation of hydroxyl ions from the intracanal medicament placed in both intact and resorbed teeth was time dependent. It was also seen that hydroxyl ion diffusion from calcium hydroxide medicament during retreatment was dependent on the type of sealer used during primary endodontic treatment.

**Key Words: $Ca(OH)_2$, pH, resorbed teeth, mineralization, retreatment**

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# LIST OF ABBREVATIONS

| S.NO | ABBREVATIONS | |
|---|---|---|
| 1. | $Ca(OH)2$ | Calcium hydroxide |
| 2. | ZnOE | Zinc Oxide Eugenol Sealer |
| 3. | MTA | Mineral Trioxide Aggregate |
| 4. | NaOCL | Sodium Hypochlorite |
| 5. | EDTA | Ethylenediaminetetraacetic Acid |
| 6. | CHX | Chlorhexidine |
| 7. | CEJ | Cemento Enamel Junction |
| 8. | CaO | Calcium oxide |
| 9. | GP | Gutta Percha |
| 10. | IRM | Intermediate Restorative Material |
| 11. | RCT | Root Canal Treatment |
| 12 | pH | Potential of Hydrogen |
| 13 | RVG | Radiovisiography |
| 14 | SPSS | Statistical Package for Social Science |
| 15 | S.D. | Standard Deviation |

# *Introduction*

# INTRODUCTION

Success of primary endodontic treatment and re-treatment is directly dependent on achieving the Schilder's biological objective. This objective aims at elimination of bacteria and its byproducts from the root canal system. Disinfection can be achieved by proper shaping followed by cleaning the canals with the help of irrigants and intracanal medicaments. Intracanal medication plays a vital role in the reduction of microbial load. The most commonly used intracanal medicament is calcium hydroxide since its introduction by Hermann in 1920. [41]

Calcium hydroxide gained clinical importance because of its ability to induce hard-tissue deposition and its efficient antibacterial properties. Siqueira and Lopes reported that the antimicrobial activity of calcium hydroxide is due to the release of hydroxyl ions in an aqueous environment. Hydroxyl ions are free radicals that show extreme reactivity with several biomolecules.[29]

The pH of calcium hydroxide ranges from 12.5 to 12.8 providing a highly alkaline environment. Antimicrobial activity of calcium hydroxide is achieved by damage to DNA, cytoplasmic membranes and by protein denaturation.[29] The high pH of Calcium hydroxide alters the integrity of the cytoplasmic membrane by chemical injury to the organic components or by saponification reaction resulting in the destruction of phospholipids and unsaturated fatty acids in the cytoplasmic membrane. Estrela et al. found that bacterial enzymatic inactivation was irreversible under extreme conditions of pH for a long period of time [12]

In re-treatment cases, Zehnder suggested calcium hydroxide suspensions and 2% CHX gel as topical disinfectants. Among them calcium hydroxide was the first choice because of its good compatibility with sodium hypochlorite and its alkaline capacity which is responsible for the antimicrobial effect and tissue dissolving capacity.[52]

The hydroxyl group is the most important component of $Ca(OH)_2$ which encourages repair and active calcification. The induced alkaline pH, not only neutralizes lactic acid from osteoclasts, thereby preventing dissolution of the mineral components of dentine, but also activates alkaline phosphatase that plays an important role in hard-tissue formation. The pH necessary for the activation of alkaline phosphatase varies from 8.6 to 10.3. [29]

Several experiments have been conducted to assess the pH changes after placement of calcium hydroxide intracanal medicament. Tronstad et al. showed that pH was greater in circumjacent dentine than peripheral dentine. Similarly, Newrich et al demonstrated difference of pH in cervical dentin and apical regions after application of calcium hydroxide intracanal medicament. [41]

The rate of diffusion of calcium hydroxide is dependent on several factors like the vehicle used with calcium hydroxide powder, duration of medicament placed in the canals, the buffering action of dentin and the residual root filling material in dentinal tubules in case of retreatment. [24]

Vehicles used with calcium hydroxide can be either Water-soluble vehicle, Viscous vehicles or Oil-based vehicles. To increase the ionic dissociation the viscosity must be lowered and to maintain the action in desired area for prolonged interval, higher molecular weight vehicles can be used with calcium hydroxide. Aqueous solutions promote rapid ion release whereas viscous vehicles release calcium and hydroxyl ions more slowly and for longer periods. Oily vehicles have limited applications as they are difficult to remove and leave a residue on the canal walls.[29]

Sjogren et al. demonstrated that application of calcium hydroxide medicament for 7 days was sufficient to reduce intracanal bacteria to a level that gave a negative culture. Shuping et al. showed that Calcium hydroxide medicament placement for at least 1 week left 92.5% of canals bacteria free.[29] Esberard et al. reported the pH in cavities on the root surface increased to more than pH 9.5 within three days, over the next 18 days showed a small decline to pH 9 and by 120 days showed about pH 10 without paste replacement. [11]

Antibacterial effect of calcium hydroxide is observed in the root canals as long as their alkaline pH is maintained. Thus, calcium hydroxide needs to diffuse through dentin to exert maximum action. But this alkalinity may be buffered by proton donors in the hydrated layer of hydroxyapatite as they provide additional protons. Diffusion of hydroxyl ions along the dentinal tubule may be lowered due to their adsorption into the hydrated layer.[30]

During the retreatment, the openings of dentinal tubules may be obstructed by the remaining sealer and gutta-percha, impacting the diffusion of hydroxyl ions through dentinal tubules.[24] Endodontic literature confirms that root canal sealers cannot be completely removed irrespective of the technique used as they may vary in their mechanism of adhesion to dentin and the depth of penetration into the dentinal tubules.[10]

Hard tissue resorption takes place in an acidic pH. Ca(OH)2, due to its alkaline pH reduces the osteoclastic activity and stimulates repair in resorptive areas. Tronstad et al. showed that the phenomenon of pH change towards the periphery is increased in areas where resorption has exposed dentine.[29]

This study evaluates the diffusion of hydroxyl ions from calcium hydroxide paste in intact and simulated resorbed roots before endodontic treatment, followed by assessing the effect of different sealers remaining, on hydroxyl ion diffusion during retreatment.

The null hypothesis was that (i) the absence of cementum in resorbed areas of teeth do not cause change in diffusion of hydroxyl ions and (ii) the rate of hydroxyl ion diffusion from calcium hydroxide medicament is not dependent on the type of sealer being used in retreatment cases.

# *Aim and Objectives*

# AIM AND OBJECTIVES

**AIM:**

The aim of this study was (i) to evaluate the hydroxyl ion diffusion from calcium hydroxide intracanal medicament placed in intact and resorbed teeth during primary endodontic treatment and retreatment and (ii) to assess if the hydroxyl ion diffusion from calcium hydroxide medicament during retreatment was dependent on the type of sealer used during primary endodontic treatment.

## OBJECTIVE:

1) To estimate invitro, **the rate of hydroxyl ion diffusion** by measuring the change in pH, from calcium hydroxide intracanal medicament (RC cal) placed **in decoronated intact roots**, into deionized water using pH meter for a period of 28 days.
2) To estimate invitro, the **pH change** due to calcium hydroxide intracanal medicament (RC cal) placed **in decoronated simulated resorbed roots**, into deionized water using pH meter for a period of 28 days.
3) To evaluate the **pH change in intact teeth on 3rd, 7th, 14th, 21st and 28th day** during primary endodontic treatment.
4) To evaluate the **pH change in simulated resorbed teeth on 3rd, 7th, 14th, 21st and 28th day** during primary endodontic treatment
5) To **compare the pH change** in **intact and resorbed teeth on 3rd, 7th, 14th, 21st and 28th day during primary endodontic treatment.**

6) To estimate the **pH change** from calcium hydroxide intracanal medicament placed **in intact teeth during retreatment** on $3^{rd}$, $7^{th}$, $14^{th}$, $21^{st}$ and $28^{th}$ day irrespective of the sealer.
7) To estimate the **pH change** from calcium hydroxide intracanal medicament placed **in simulated resorbed teeth during retreatment** on $3^{rd}$, $7^{th}$, $14^{th}$, $21^{st}$ and $28^{th}$ day irrespective of the sealer.
8) To evaluate the **pH change in intact teeth on $3^{rd}$, $7^{th}$, $14^{th}$, $21^{st}$ and $28^{th}$ day** during retreatment.
9) To evaluate the **pH change in simulated resorbed teeth on $3^{rd}$, $7^{th}$, $14^{th}$, $21^{st}$ and $28^{th}$ day** during retreatment
10) To compare the **pH change** in **intact and resorbed teeth** on **$3^{rd}$, $7^{th}$, $14^{th}$, $21^{st}$ and $28^{th}$ day during retreatment.**
11) To compare the pH change between intact and simulated resorbed teeth, **during primary endodontic treatment and retreatment** on $3^{rd}$, $7^{th}$, $14^{th}$, $21^{st}$ and $28^{th}$ day irrespective of the sealer used.
12) To compare invitro, the pH changes due to calcium hydroxide intracanal medicament placed **during retreatment** in intact teeth and simulated resorbed teeth, **after retrieval of GP and root canal sealer**, viz., ZnOE sealer, Sealapex, MTA fillapex or BioRoot RCS, into deionized water using pH meter, during the entire period of study.
13) To compare the **pH change during retreatment on $3^{rd}$, $7^{th}$, $14^{th}$, $21^{st}$ and $28^{th}$ day** after placement of calcium hydroxide medicament

following removal of ZnOE, Sealapex, MTA fillapex or BioRoot RCS as sealer in intact teeth and simulated resorbed teeth.

14) To compare the **pH change on 3$^{rd}$, 7$^{th}$, 14$^{th}$, 21$^{st}$ and 28$^{th}$ day in intact teeth during retreatment** after placement of calcium hydroxide intracanal medicament following removal of sealer.

15) To compare the **pH change on 3$^{rd}$, 7$^{th}$, 14$^{th}$, 21$^{st}$ and 28$^{th}$ day in simulated resorbed teeth during retreatment** after placement of calcium hydroxide intracanal medicament following removal of sealer.

# *Review of Literature*

**Tronstad L et al (1981)[50]** studied the pH changes of dental tissues in monkeys after endodontic treatment with calcium hydroxide. A pH of 6.0 to 7.4 was noted in the pulp, dentin, cementum, and periodontal ligament of untreated teeth with pulpal necrosis. Replanted and non-replanted teeth with completed root formation showed pH values of 8.0 to 11.1 in the circumpulpal dentin, and 7.4 to 9.6 in the more peripheral dentin in teeth with incomplete root formation when treated with calcium hydroxide, the entire dentin showed a pH of 8 to 10. The pH of the cementum was not influenced by the calcium hydroxide. However, in resorption areas, an alkaline pH was also observed at the exposed dentinal surfaces.

**Pashley D.H et al (1986)[33]** evaluated the effects of Calcium Hydroxide on Dentin Permeability. Topical application of Ca(OH)2 paste to the smear layer reduced dentin permeability further, to levels 48% below that of untreated smear layers. When the Ca(OH)2-treated smear layers were exposed to 6% citric acid for two min, dentin permeability returned to the initial acid-etched value. Thus, Ca(OH)2 is effective at reducing the permeability of both the smear layer and of acid-etched dentin, in vitro.

**Tao L et al (1991)[46]** quantitated the sequential effects of endodontic procedures on the permeability of human root dentin in vitro. The study concluded that EDTA treatment inside the instrumented canal to remove the smear layer did not increase permeability significantly.

**Foster KH et al (1993)[15]** evaluated the effect of smear layer removal on the diffusion of calcium hydroxide through radicular dentin. The results demonstrated that CH diffuses from the root canal to the exterior surface of the root and that the removal of the smear layer may facilitate this diffusion.

**Friedman S et al (1993)[16]** evaluated the efficacy of ultrasonic retreatment in canals obturated with single-cone gutta-percha and Ketac-Endo. The study concluded that ultrasonic retreatment may be performed effectively in root canals obturated with single-cone gutta-percha and Ketac-Endo.

**Nerwich A et al (1993)[30]** measured the pH changes in the root dentin over a period of 4 weeks. pH was measured in the apical and cervical third of the inner and outer root dentin at various time intervals. In the inner dentin, the pH increased within hours showing a peak value of 10.8 cervically and 9.7 apically. Whereas, in the outer root dentin, pH rise was noted only after 1 to 7 days and approximately after 2 to 3 weeks peak levels of pH 9.3 cervically and 9.0 apically were recorded. The results show that hydroxyl ions diffuse through root dentin. Diffusion was faster and reached higher levels cervically than apically. Surface pH measurements showed that hydroxyl ions diffusion is limited in intact root surface.

**Estrela C et al (1995)[12]** analyzed and discussed the mechanism of action of calcium and hydroxyl ions on anaerobic bacteria, starting from the isolated study of the influence of pH on these bacteria, as well as the mechanism of action of calcium hydroxide on tissue.

**Esberard RM et al (1996)[11]** investigated long-term pH changes in cavities prepared in root surface dentin of extracted teeth after obturation of the root canal with gutta-percha and a variety of sealers containing calcium hydroxide. Each of four groups was obturated with gutta-percha and either Sealapex, Sealer 26, Apexit, or CRCS, all of which contain calcium hydroxide. The study concluded that calcium hydroxide-containing cements, although suitable for use as root canal sealants, do not produce an alkaline pH at the root surface. If such a pH change is related to treatment of root resorption, these sealants do not contribute to this treatment.

**Kouvas V et al (1998)[26]** examined the effect of the smear layer on the penetration depth of Sealapex, Roth 811, and CRCS root canal sealers into the dentinal tubules. The teeth were then randomly divided into 2 groups. The smear layer was removed from all teeth in group A with EDTA and NaOCl. The smear layer remained in all teeth in group B. The study concluded that the presence of smear layer obstructed the penetration of all sealers into the dentinal tubules.

**Siqueira JF et al (1999)[41]** reviewed the primary function and physicochemical properties of calcium hydroxide as a routine intracanal medicament

**Safavi K et al (2000)[37]** evaluated the influence of mixing vehicle on dissociation of calcium hydroxide in solution. The study concluded that concluded that use of non-aqueous mixing vehicles may impede the effectiveness of calcium hydroxide as a root canal dressing.

**Hosaya et al (2001)[20]** determined the concentration of calcium and pH in the periapical region after application of 1 of 4 different calcium hydroxide preparations into experimental root canals. The highest calcium concentration was observed after 3 days for the mixture groups, whereas that for the powder only group was found at 7 days. Peak pH change was found after 14 days for the mixture groups, whereas that for the powder only group was found at 49 days. These results suggest that the time required for optimum intracanal activity when using calcium hydroxide mixtures is at least 2 weeks.

**Evans M et al (2002)[14]** clarified the mechanisms that enable E. faecalis to survive the high pH of calcium hydroxide. The study concluded that Survival of E. faecalis in calcium hydroxide appears to be unrelated to stress induced protein synthesis, but a functioning proton pump is critical for survival of E. faecalis at high pH.

**Rajput et al (2004)[34]** compared the apical microleakage of vitapex (calcium hydroxide based paste) when used with single gutta percha cone with that of dentalis KEZ (calcium hydroxide and zincoxide eugenol based sealer) and zinc oxide eugenol sealer when used with laterally condensed gutta percha obturation technique. Results of this study showed that calcium hydroxide based endodontic material leaked comparatively less as compared to Zinc Oxide Eugenol sealer. Vitapex with single gutta-percha cone provided an adequate apical Seal against dye penetration.

**Christopher P et al (2004)[6]** Enterococcus faecalis tolerates highly alkaline environments, yet the exact pH required for killing E. faecalis is not known. This study tests growth at 0.5 increments from pH 9.5 to 12. Results proved no growth occurred in any of the pH 11.5 or pH 12 tubes. Apparently, pH 10.5 to 11.0 retards growth of E. faecalis, whereas no tubes showed growth at pH 11.5 or greater.

**Hülsmann M et al (2004)[21]** evaluated the efficacy, cleaning ability and safety of three different rotary nickel-titanium instruments FlexMaster, GT Rotary, ProTaper and Hedström files with and without a solvent (eucalyptol) versus hand files in the removal of gutta-percha root fillings. The study concluded that FlexMaster and ProTaper NiTi instruments proved to be efficient and time-saving devices for the removal of gutta-percha. The use of eucalyptol as a solvent shortened the time to reach the working length and to remove the gutta-percha, but this was not significant.

**Teixeira CS et al (2005)[48]** verified the influence of irrigation time with EDTA and NaOCl on intracanal smear layer removal. The study concluded that anal irrigation with EDTA and NaOCl for 1, 3 and 5 min were equally effective in removing the smear layer from the canal walls of straight roots.

**Mamootil K et al. (2007)[27]** compared the depth and consistency of penetration of three different root canal sealer cements into dentinal tubules in extracted teeth and also measured the penetration of an epoxy resin-based sealer cement in vivo using scanning electron microscopy. Results showed that AH26 demonstrated the deepest penetration, followed by EndoREZ and Pulp Canal Sealer EWT. In the clinical cases, all teeth demonstrated sealer penetration to varying depths (98–1490 µm). Hence, it was concluded that the depth and consistency of dentinal tubule penetration of sealer cements appears to be influenced by the chemical and physical characteristics of the materials.

**Sherma Saif et al (2008)**[38] measured hydroxyl ion diffusion through dentinal tubules into a bathing solution. The study concluded that final canal irrigation with 3 mL 17% EDTA and 10 mL 6% NaOCl before Ca(OH)2 placement allowed the greatest hydroxyl ion diffusion to the root surface.

**Taşdemir T et al (2008)**[47] investigated the ability of three rotary nickel-titanium instruments and hand instrumentation to remove gutta-percha and sealer. he teeth were randomly divided into four groups of 15 specimens each. Removal of gutta-percha was performed with the following devices and techniques: ProTaper, R-Endo, Mtwo and Hedström files. The study concluded that ProTaper left significantly less gutta-percha and sealer than Mtwo instruments. Complete removal of materials did not occur with any of the instrument systems investigated.

**Gu LS et al (2008)**[18] evaluated the efficacy of the ProTaper Universal rotary retreatment system for gutta-percha (GP) removal from root canals. The study concluded that the ProTaper Universal rotary retreatment system proved to be an efficient method of removing GP and sealer from maxillary anterior teeth.

**Duncan HF et al (2008)[10]** reviewed the management of commonly encountered root filling materials during non-surgical re-treatment, including the clinical procedures necessary for removal and the associated risks.

**Zehnder et al (2008)[52]** reviewed the differences between primary root canal treatments and re-treatments and explored in view of optimal disinfection of the root canal system.

**Ng YL et al (2008)[31]** investigated the effects of study characteristics on the reported success rates of secondary root canal treatment and the effects of clinical factors on the success of re RCT. The pooled estimated success rate of secondary root canal treatment was 77%.

**Keles A et al (2009)[25]** compared the ability of ethylene diamine tetra acetic acid and sodium hypochlorite solutions to dissolve sealers. The solubility of six sealers—calcium hydroxide, polyketone, zinc oxide–eugenol, silicone and two epoxy resins—in EDTA and two concentrations of NaOCl were assessed. The study concluded that during nonsurgical endodontic re-treatment, EDTA and NaOCl solutions used for removing smear layer aided in the retreatment by dissolving some root canal sealers.

**Torabinejad M et al (2009)[49]** compared the clinical and radiographic outcomes of nonsurgical retreatment with those of endodontic surgery to determine which modality offers more favorable outcomes. It was concluded that endodontic surgery offers more favorable initial success, but nonsurgical retreatment offers a more favorable long-term outcome.

**Ordinola-Zapata R et al (2009)[32]** compared the percentage and depth of sealer penetration into dentinal tubules during obturation using Sealer 26, GuttaFlow, or Sealapex in root canals filled with the lateral compaction technique. The study concluded that although Sealapex displayed deeper penetration into the dentinal tubules there was no difference in the percentage of adaptation to the root canal walls among the 3 sealers evaluated.

**Horvath SD et al (2009)[19]** determined the influence of solvents on gutta-percha and sealer remaining on root canal walls and in dentinal tubules. It was concluded that solvents led to more gutta-percha and sealer remnants on root canal walls and inside dentinal tubules.

**Takahashi CM (2009)[45]** evaluated the efficacy of a nickel-titanium rotary instrument system with or without a solvent versus stainless steel hand files for gutta-percha removal. The study concluded that all of the techniques proved helpful for the removal of endodontic filling material, and they were similar in material remaining after retreatment, but the ProTaper Universal rotary retreatment system without chloroform was faster.

**Roggendorf MJ et al (2010)[35]** assessed the efficacy of removing Activ GP or GuttaFlow from canals using NiTi instruments. The study concluded that Both root fillings with ActivGP and GuttaFlow were removed with nickel-titanium rotary instruments. Enlargement of canals up to two sizes beyond the pre-retreatment size was necessary to minimize the amount of sealer remaining.

**Roghanizad N et al (2011)[36]** investigated pH changes on the root surface following treatment with calcium-hydroxide intracanal medicament in intact and resorbed roots over a 4 weeks period. Results showed an average increase in pH values at all time periods during the study, except a mild decline at 7 days and 14 days. It was also shown that diffusion of hydroxyl ions was similar in both resorbed and intact roots.

**Mohammadi Z et al (2011)[29]** reviewed the properties and clinical applications of calcium hydroxide in endodontics and dental traumatology including its antibacterial activity, antifungal activity, effect on bacterial biofilms, the synergism between calcium hydroxide and other agents, its effects on the properties of dentine, the diffusion of hydroxyl ions through dentine and its toxicity.

**Balguerie E et al (2011)[3]** assessed the tubular adaptation and penetration depth and the adaptation to the root canal walls in the apical, middle, and coronal third of the root canal of 5 different sealers used in combination with softened gutta-percha cones. The study concluded that the tubular penetration and adaptation varies with the different physical and chemical properties of the sealers used.

**Fulzele P et al (2011)**[17] estimated the calcium ion and hydroxyl ion release and pH levels of calcium hydroxide based products, namely, RC Cal, Metapex, calcium hydroxide with distilled water, along with the new gutta-percha points with calcium hydroxide. Based on results it was concluded that Calcium hydroxide with sterile water and RC Cal pastes liberate significantly more calcium and hydroxyl ions and raise the pH higher than Metapex and calcium hydroxide gutta-percha points.

**Borges RP et al (2012)**[4] compared the changes in the surface structure and elemental distribution, as well as the percentage of ion release, of four calcium silicate-containing endodontic materials with a well-established epoxy resin based sealer, submitted to a solubility test. It was concluded that AH Plus and MTA-A were in accordance with ANSI/ADA's requirements. High levels of Ca2+ ion release was observed in all materials except AH Plus.

**Buch A et al (2012)**[5] compared the pH changes following intracanal placement of Conventional Calcium Hydroxide Paste - Ca(OH)2 + Distilled Water, Commercial Ca(OH)2 Paste - (RC Cal) and Ca(OH)2 Gutta Percha Points (Hygenic) over different time intervals from hours to 14 days to evaluate which of these products has a better alkalinizing potential over time to be used as an inter-appointment intracanal dressing. Results signified conventional calcium hydroxide paste group showed a rapid rise in pH immediately upon placement and the pH continued to rise to around 11.66 for the entire time period of 14 days. whereas, the commercial paste showed initial readings almost similar to the conventional paste group till 48 hours after which it declined significantly and reached to a bottom value of 7.65 at 14th day.

**Dash A K et al (2012)**[8] evaluated and compared calcium concentration and pH of the periapical environment after applying calcium hydroxide into root canals. The results suggested that the time required for optimum intracanal activity when using calcium hydroxide mixtures is at least 1 week.

**Jayasudha et al (2012)**[23] tested the pH changes that occurred over a period of 7 days using calcium hydroxide, Metapex and Ledermix & Calcium hydroxide combination and to compare the effect of contamination in a simulated periapical environment. Results disclosed that Calcium hydroxide significantly increased the pH of the surrounding medium with and without contamination of root canals compared to combination medicaments. Gradual change in Ph of calcium hydroxide was recorded with a mean pH value of 7.50±0.02 to 11.23 for 7 days.

**Kazemipoor M et al (2012)**[24] compared pH changes in retreated and non- retreated teeth at the cervical, middle and apical surfaces of root dentin, after canal obturation with two different calcium hydroxide pastes. Results showed little pH changes in retreated teeth is because the hydroxyl ions were unable to penetrate into the dentinal tubules. Thus, to achieve higher pH at the root surface in retreated teeth, it was shown that more dentin must be removed from the inner walls. Use of normal saline as a vehicle for calcium hydroxide rather than acidic pH materials also facilitated increase in pH.

**Estrela C et al (2014)**[13] discussed the relevant factors associated with patient's health, tooth and dentist that could account for a successful RCT.

**Iriboz E et al (2014)**[22] evaluated the effectiveness of the ProTaper and Mtwo retreatment systems for removal of resin-based obturation techniques during retreatment. Resilon + Epiphany, gutta-percha + Epiphany, gutta-percha + AH Plus and gutta-percha + Kerr Pulp Canal Sealer (PCS) combinations were used for obturation. The study concluded that although ProTaper retreatment files worked faster than did Mtwo retreatment files in terms of removing root canal obturation materials, both retreatment systems are effective, reliable and fast.

**Dos Santos LG et al (2014)**[9] evaluated the diffusion of hydroxyl ions from calcium hydroxide paste (CH) before root canal filling and after retreatment. The root canals were divided into five groups and filled with Resilon/Real Seal (G1) or gutta-percha and Endofill (G2), Sealapex (G3), AH Plus (G4) or MTA Fillapex (G5) sealers. The study concluded that Hydroxyl ions are able to diffuse through dentinal tubules. Regardless of the filling material, it was possible to re-establish the permeability of dentine to ionic diffusion after retreatment. Time had a positive influence on ionic diffusion.

**Amoroso Silva et al (2014)**[2] analyzed the quality of obturation and physical properties of MTA Fillapex and AH Plus sealer using confocal laser-scanning microscope (CLSM). The CLSM analysis showed that MTA Fillapex exhibited the highest percentage of gaps at all sections. Physical tests revealed adequate properties for both sealers except for a higher solubility of the MTA Fillapex. The MTA Fillapex presented flowability and intratubular penetration similar to the AH Plus.

**Swati Srivastava et al (2014)[44]** evaluated invitro, the pH and calcium ion diffusion from MTA Fillapex and Sealapex through simulated external root resorption. The groups were group I, MTA Fillapex (Angelus) and in group II, Sealapex (Sybron Endo) was used as root canal sealer. In group III, pulp space was not filled with any material. The study concluded that Sealapex provided highest pH and calcium release as compared to other groups ($p < 0.001$).

**Silva R. V et al (2015)[40]** evaluated the filling effectiveness and dentinal penetration of the sealers AH Plus, Pulp Canal Sealer EWT, Sealapex and MTA Fillapex applied according to the vertical condensation technique using thermoplastic gutta-percha and analyzed by stereomicroscopy and confocal laser scanning microscopy. Results showed that the penetration into dentinal tubules at 4 and 6 mm Pulp Canal Sealer EWT had an inferior performance compared to MTA Fillapex and AH Plus.

**Sonu et al (2016)[43]** tested the dentinal tubule penetration of MTA Fillapex, GuttaFlow® 2 sealer with standard sealer AH Plus in instrumented root canals obturated by using cold lateral compaction techniques in either the presence or absence of the smear layer. It was concluded that the removal of smear layer AH plus sealer showed deeper penetration into the dentinal tubules at cervical and middle third of root compared with apical third.

**Mcmichael et al (2016)**[28] measured the tubule penetration of EndoSequence BC Sealer, QuickSet2, NeoMTA Plus and MTA Fillapex sealers during continuous wave and single-cone obturation techniques. It was concluded that both techniques produced similar tubule penetration at both 1-mm and the 5-mm levels with the tricalcium silicate sealers BC Sealer, QuickSet2, and NeoMTA Plus.

**Viapiana et al (2016)**[51] investigated the ability of BioRoot RCS and AH Plus to effectively fill the root canals by using lateral compaction technique. The percentage of voids, sealing ability, interaction of sealer with dentine, and sealer penetration were assessed. Results revealed that BioRoot RCS exhibited significantly more percentage of voids than AH Plus. BioRoot RCS exhibited a different pattern of sealer penetration and interaction with the dentine walls compared to AH Plus. It was concluded that BioRoot RCS exhibited higher void volume than AH plus sealer.

**Sonali GAK et al (2017)**[42] compared the calcium ion, hydroxyl ion release and pH levels between nano calcium hydroxide and other calcium hydroxide based intracanal medicaments in vitro. Calcium hydroxide based substances were divided into six groups: Group A (Calcium hydroxide powder with distilled water), Group B (Nano calcium hydroxide powder with distilled water), Group C (Vitapex), Group D (RC Cal), Group E (Dentocal) & Group F (Calcium hydroxide points). The study concluded that Aqueous based preparations of calcium hydroxide should be chosen over points or oil-based calcium hydroxide preparations.

**Cruz et al (2017)**[7] evaluated the effect of a calcium hydroxide dressing on the tubular penetration of two endodontic sealers, AH Plus and MTA Fillapex. Results revealed calcium hydroxide dressing did not interfere with the apical penetration of both tested sealers. Overall, MTA Fillapex presented higher tubular penetration than AH Plus obturations.

**Alyahya A et al (2017)**[1] showed the importance of an intact layer of cementum on the root surface in preventing bacterial penetration. Results revealed that the absence of cementum facilitates bacterial penetration into dentinal tubules. It also suggests that the process of radicular dentin infection is time dependent and highlights the importance of early treatment of infected teeth, especially in situations in which cementum discontinuity is suspected.

**Siboni F et al (2017)**[39] evaluated the chemical and physical properties of a tricalcium silicate root canal sealer containing povidone and polycarboxylate (BioRoot RCS), a calcium silicate MTA-based sealer containing a salicylate resin (MTA Fillapex), a traditional eugenol-containing sealer (Pulp Canal Sealer) and an epoxy resin-based root canal sealer (AH Plus). The study concluded that BioRoot RCS had bioactivity with calcium release, strong alkalizing activity and apatite-forming ability, and adequate radiopacity.

# *Materials and Methods*

# MATERIALS AND METHODS

The present invitro study was carried out in the Department of Conservative Dentistry and Endodontics, Ragas Dental College and Hospital, Chennai.

## ARMAMENTARIUM

1. 40 Extracted human mandibular single rooted premolars.
2. Airotor highspeed handpiece [NSK, Japan].
3. Round Diamond burs [Mani Inc Japan].
4. K files # 15 - # 40 - 21mm [Mani Inc, Japan].
5. Protaper Universal rotary files [Densply Maillefer].
6. Protaper Universal Retreatment Files [Densply Maillefer]
7. NSK Endomotor [Densply Maillefer]
8. 2 ml disposable Syringes [Hindustan syringes & Medical Services Ltd].
9. 17% EDTA solution [Prevest DenPro].
10. Sodium hypochlorite 3% [ Prime Dental Products PVT].
11. Saline [ Prime Dental Products PVT]
12. Irrisafe ultrasonic irrigation tip [Patterson Dental]
13. Acrylic material Clear cold cure [Dental products of India LTD]
14. RC Cal [Prime Dental Products PVT]
15. Deionised water [Emplura, Merck specialities, Mumbai]
16. BioRoot RCS [Septodont, USA]
17. MTA Fillapex [Angelus, Brazil]
18. Sealapex [Kerr Sybron Endo, USA]

19. Spreaders-21mm [Mani Inc, Japan]
20. 2% GP Points [Densply Maillefer]
21. IRM [Densply Maillefer]
22. Varnish [GRN Cellulose Pvt Ltd, Varnasi]
23. Digital pH meter [MxRady Lab shop, Delhi]
24. 30 ml plastic containers

## METHODOLOGY

### SAMPLE SIZE:

A total of 40 extracted human mandibular single rooted premolars were selected [Fig 1].

### SELECTION AND PREPARATION OF THE SAMPLES:

Mandibular premolars with single root canal, no calcifications, and the absence of a complicated root canal anatomy were collected, cleaned and stored in saline with 0.1% thymol. The teeth were decoronated using a diamond disc and the roots were standardized to 14 mm in length [Fig 2]. The working length was established using size # 15 K-file (Mani) and was kept 1mm short of this length. The apical third of the roots was sealed with cold cure acrylic material to standardize all teeth. Root canals were prepared using Protaper universal rotary instruments (Dentsply, India) up to apical size F3 [Fig 13] and ensured with radiographs [Fig 18 a]. The canals were irrigated with 2ml of 3% NaOCl and 5 ml of 17% EDTA liquid after each instrumentation. Final irrigation was done with 5 ml of Saline [Fig 14].

**EXPERIMENTAL GROUPS:**

After the instrumentation, the specimens were randomly divided into two groups of 20 teeth each. (N=20)

**Group 1:** Intact roots

**Group 2:** Simulated resorbed roots

**PREPARATION OF SIMULATED ROOT RESORPTION**

Two wells were drilled in cervical and middle third on the buccal and lingual root surfaces of all teeth in group 2, using a # 008 highspeed bur. The cavities were 1 mm deep and 1.5 mm in diameter [Fig 12]. The teeth were then thoroughly rinsed with 5 ml of 17% EDTA and 5 ml of 3% sodium hypochlorite for 1 minute to remove the smear layer, then rinsed with saline.

**PLACEMENT OF CALCIUM HYDROXIDE INTRACANAL MEDICAMENT**

All the teeth were dried with paper points. RC Cal was introduced into the canals directly via the delivery tip provided by the manufacturer. The tip was placed into the canal until resistance was felt, simultaneously while injecting the medicament into the canal, the tip was slowly withdrawn until the whole canal was filled with calcium hydroxide [Fig 15]. The complete and uniform placement of RC Cal was ensured with radiographs [Fig 18 b]. Coronal access cavities were sealed with IRM and coated with a layer of varnish. The teeth were then placed in plastic containers containing 10 mL of deionized water and incubated at 37ºC.

**pH MEASUREMENT DURING PRIMARY ENDODONTIC TREATMENT**

To evaluate the rate of hydroxyl ion diffusion from calcium hydroxide intracanal medicament, a digital pH meter was immersed into the containers having deionized water. Readings were obtained from the LCD display in the pH meter (with accuracy of 0.1 per pH unit) [Fig 17]. The pH values were recorded on 3, 7, 14, 21 and 28 days and documented in Microsoft excel sheet.

**SUB GROUPS**

After the measuring the pH changes during primary endodontic treatment, samples in both the experimental groups were further subdivided into 4 sub-groups of 10 teeth each (N=10), based on the sealer used for obturation.

**Group A:** Zinc oxide eugenol sealer

**Group B:** Sealapex

**Group C:** MTA Fillapex

**Group D:** BioRoot RCS

The teeth were removed from their respective containers, the calcium hydroxide intracanal medicament was removed by copious irrigation with 3% NaOCl and 17% EDTA and ultrasonic activation using irrisafe ultrasonic tip [Fig 6]. Final irrigation was done with 5ml of Saline. The root canals were then dried with paper points and the teeth in each subgroup were obturated with the respective root canal sealers and 2% gutta-percha points by lateral condensation technique

[Fig 18 c]. Temporary coronal seal was provided with IRM and varnish coating. The specimens were stored for 7 days.

**RETREATMENT PROTOCOL**

The teeth were taken out of their respective containers and dried completely. The coronal seal was removed using airotor and round bur. Then the retreatment procedure was performed with protaper universal retreatment files (Dentsply Maillefer) D1, D2, D3, with brushing movements against the canal walls with a constant speed of 500rpm, as recommended by the manufacturer. [Fig 16] The preparation was complete when no more obturation material was observed sticking to the retreatment instruments. The complete removal of obturation materials were confirmed with radiographs [Fig 18 d]. Root canals were irrigated in between all instrument changes using 2ml of 3% NaOCl, 2ml of 17% EDTA and 5ml of saline was used as final rinse.

**pH MEASUREMENT DURING RETREATMENT**

The root canals were then dried with paper points and calcium hydroxide intracanal medicament was placed according to the above-mentioned protocol. The pH was measured with digital pH meter at 3, 7, 14, 21 and 28 days and recorded in Microsoft Excel. pH values were compared between all groups and sub-groups.

# METHODOLOGY

40 single rooted mandibular premolars with straight canals were selected

Working length determined

Instrumentation completed with protaper universal rotary files upto F3

Irrigation with 3% sodium hypochlorite and 17% EDTA

The were divided randomly into 2 groups

Group 1 : Intact roots

Group 2 : Simulated root resorption

The canals were filled with RC cal, temporary coronal seal was given and placed in individual containers with 10ml of deionised water

pH was measured on $3^{rd}$, $7^{th}$, $14^{th}$, $21^{st}$ and $28^{th}$ day (i.e pH during primary endodontic treatment).

The teeth were then divided into 4 sub-groups of 10 teeth each based on the materials used for obturation

Group A : GP and ZnOE sealer

Group B : GP and Sealapex

Group C : GP and MTA Fillapex

Group D : GP and BioRoot RCS

After storage for 7 days root canal filling material removed using Protaper universal retreatment system

↓

Irrigation was done with 3% sodium hypochlorite and 17% EDTA followed by final rinse with saline.

↓

The canals were filled with RC cal, temporary coronal seal was given and placed in individual containers having 10ml of deionised water

↓

pH was measured on $3^{rd}$, $7^{th}$, $14^{th}$, $21^{st}$ and $28^{th}$ day (pH during retreatment).

↓

The pH readings between all groups and sub-groups were statistically analysed.

# *FIGURES*

**FIGURE 1: EXTRACTED HUMAN MANDIBULAR PREMOLARS**

**FIGURE 2: DECORONATION OF THE TEETH NEAR THE CEJ**

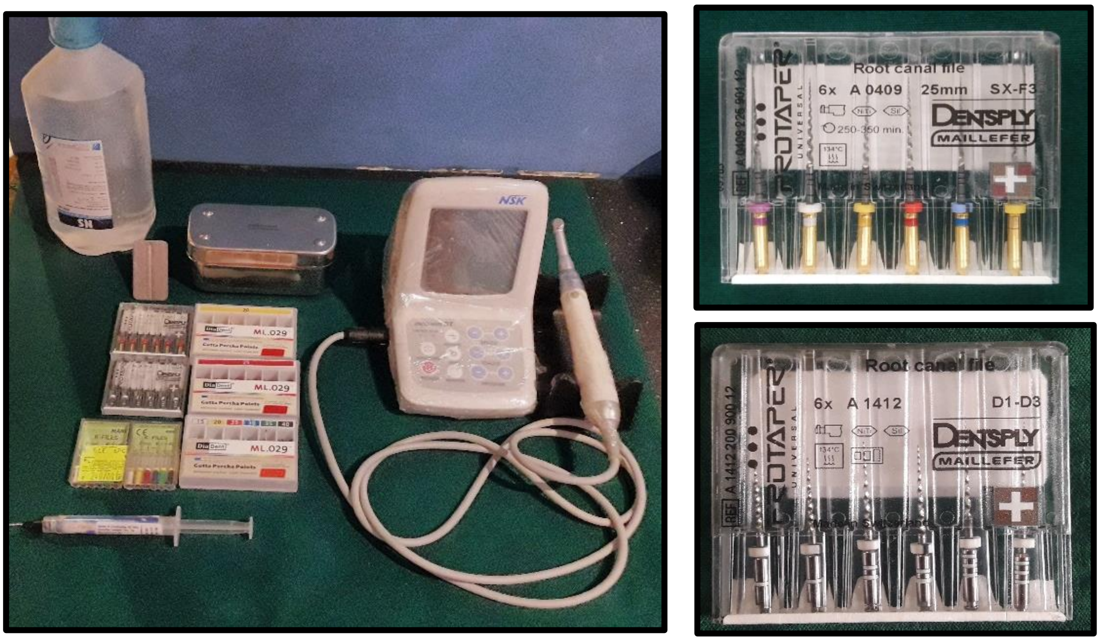



**FIGURE 3: ARMAMENTARIUM FOR ROOT CANAL TREATMENT AND RETREATMENT**


**FIGURE 4: IRRIGANTS USED**


**FIGURE 5: CALCIUM HYDROXIDE**

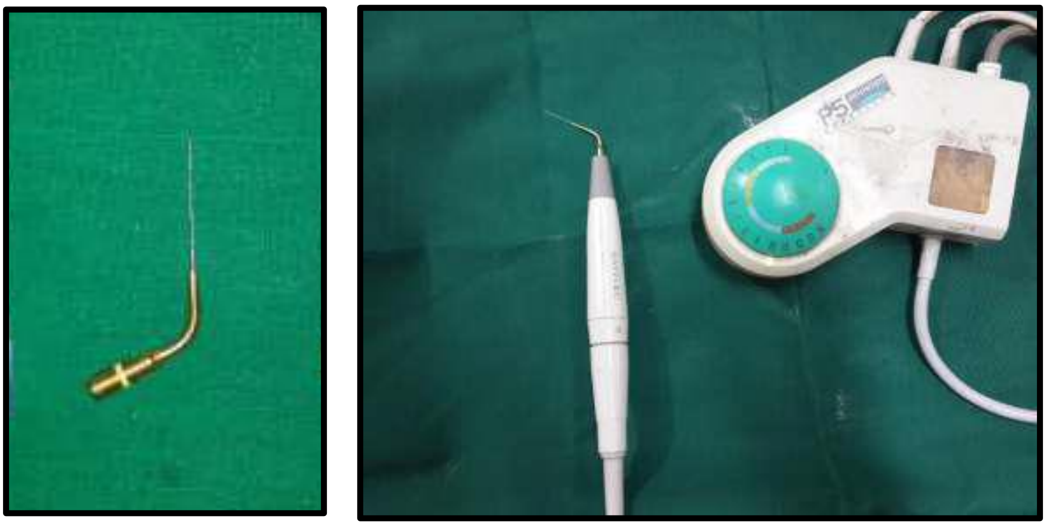


**FIGURE 6: SATELEC UNIT ATTACHED WITH IRRISAFE ULTRASONIC TIP**


**FIGURE 7: DEIONIZED WATER**


**FIGURE 8: PLASTIC CONTAINERS (30 ML)**


**FIGURE 9: DIGITAL pH METER**


**FIGURE 10: EXPERIMENTAL ROOT CANALS SEALERS**

**FIGURE 11: INCUBATOR**

**FIGURE 12: CREATION OF WELLS TO SIMULATE RESORPTIVE AREAS IN CEMENTUM**


**FIGURE 13: CLEANING AND SHAPING OF THE CANALS**


**FIGURE 14: IRRIGATION PROTOCOL (3%NAOCL & 17% EDTA)**

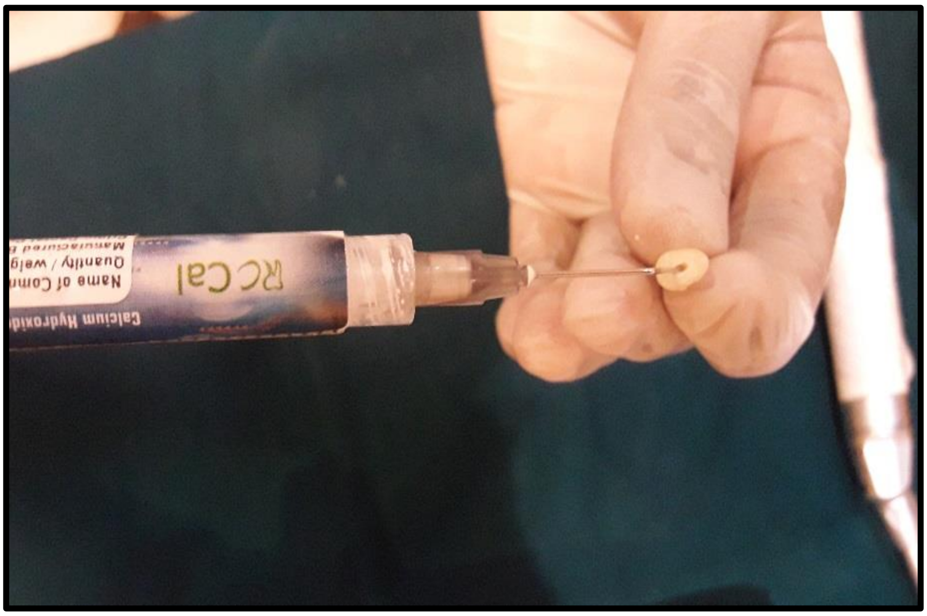
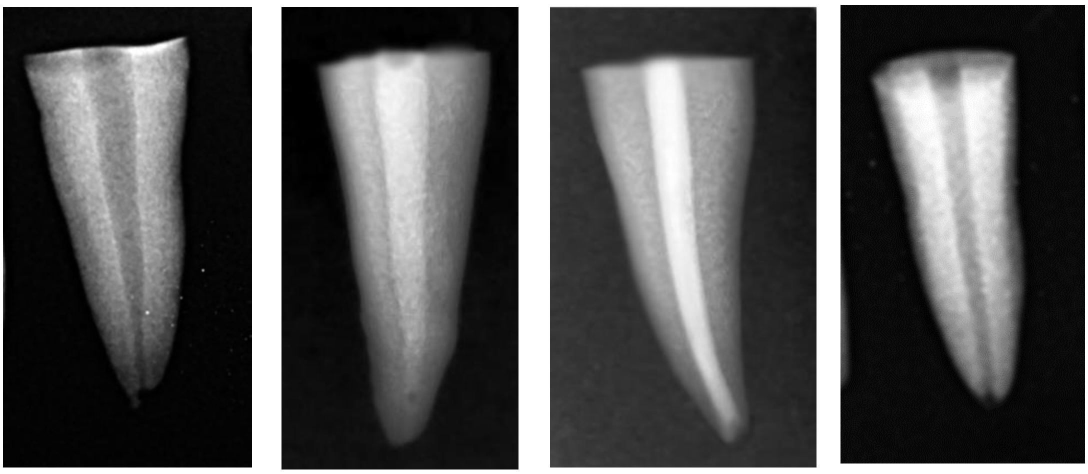


**FIGURE 15: RC CAL PLACEMENT IN THE CANALS**

**FIGURE 16: RETRIEVAL OF FILLING MATERIALS WITH PROTAPER UNIVERSAL RETREATMENT FILES**


**FIGURE 17: MEASURING pH USING pH METER**



(a) BMP done (b)$Ca(OH)_2$ dressing (c)Obturation (d) Filling retrieved

**FIGURE 18: RVG (BUCCOLINGUAL ORIENTATION)**

# *Results*

# RESULTS

**Data compilation and presentation:**

Data obtained were compiled systematically in Microsoft Excel spreadsheet. The dataset was subdivided and distributed meaningfully and presented as graphs and tables.

**Statistical analysis:**

Statistical analysis was performed using a personal computer in Statistical Package for Social Sciences software (SPSS IBM). The p value was set for 0.05 and any value equal to or less than was considered to be significant.

Normality was tested using Shapiro Wilk test and the data was not normally distributed. Kruskal-Wallis test was done to study the overall variance within groups. However, it was not possible to identify the difference between the various subgroups hence, the Post hoc Mann-Whitney was done in order to determine which groups differ from each other.

**Table 1 and Graph 1** shows the Mean and S.D. pH Changes in Intact and Resorbed Teeth During Primary Endodontic Treatment. In Group 1 (intact teeth), the mean pH values on $3^{rd}$,$7^{th}$,$14^{th}$,$21^{st}$ and $28^{th}$ day were 11.505 (±0.0945), 12.040 (±0.1046), 10.195 (±0.0826), 9.825 (±0.0910) and 9.395 (±0.0887) respectively, whereas in Group 2 (resorbed teeth) pH values were 11.625 (±0.0786), 12.345 (±0.1191), 10.125 (±0.716), 9.615 (±0.0745) and 9.25 (±0.0513) on $3^{rd}$,$7^{th}$,$14^{th}$,$21^{st}$ and $28^{th}$ day respectively.

**Table 2** shows the Statistical Comparison of pH Changes in Intact and Resorbed Teeth During Primary Endodontic Treatment on 3$^{rd}$, 7$^{th}$, 14$^{th}$, 21$^{st}$ and 28$^{th}$ day. Inter group comparison during primary endodontic treatment shows that there is significant difference between intact teeth (Group 1) and resorbed teeth (Group 2) ($p<0.05$) in rate of hydroxyl ion diffusion at all time periods. The mean pH values show that the hydroxyl ion diffusion was more in resorbed teeth (Group 2) than the intact teeth (Group 1) on 3$^{rd}$ and 7$^{th}$ day, whereas, pH values on the 14$^{th}$,21$^{st}$ and 28$^{th}$ day showed that the diffusion of hydroxyl ion was higher in the intact teeth (Group 1) than resorbed teeth (Group 2).

**Table 3** shows the Statistical Comparison of pH change on 3$^{rd}$, 7$^{th}$, 14$^{th}$, 21$^{st}$ and 28$^{th}$ day During Primary Endodontic Treatment in Intact Teeth and Resorbed Teeth. There was significant difference in pH change from day 3 to day 28 in both groups (intact and resorbed teeth) ($p=0.00$). Mean pH values showed that hydroxyl ions diffuse faster for the 1$^{st}$ three days raising the pH, marginally increases till the end of one week where the peak pH is obtained (approximately to 11 in intact teeth and 12 in resorbed teeth) and then starts reducing gradually till the 28$^{th}$ day.

**Table 4 and Graph 2** shows the Mean & S.D. pH Changes in Intact Teeth during Retreatment on 3$^{rd}$, 7$^{th}$, 14$^{th}$, 21$^{st}$ and 28$^{th}$ day. The hydroxyl ion diffusion, during retreatment of different sealers, in the decreasing order was Group A > Group B > Group C > Group D at all time intervals.

**Table 5 and Graph 3** shows the Mean & S.D. pH. Changes During Retreatment in Resorbed Teeth. The hydroxyl ion diffusion, during retreatment of different sealers, in the decreasing order was Group A > Group B > Group C > Group D at all time intervals

**Graph 4** shows the Change in pH During Retreatment in Intact and Resorbed Teeth with ZnOE as Sealer. pH of intact teeth was 10.06, 11.04, 9.16, 8.96 and 8.72 respectively on the $3^{rd}$ day, $7^{th}$ day, $14^{th}$ day, $21^{st}$ day and $28^{th}$ day. In resorbed teeth pH was 11.16, 11.52, 9.00, 8.64 and 8.62 respectively on the $3^{rd}$ day, $7^{th}$ day, $14^{th}$ day, $21^{st}$ day and $28^{th}$ day.

**Graph 5** shows the Change in pH During Retreatment in Intact and Resorbed Teeth with Sealapex as Sealer. pH of intact teeth was 10.02, 11.02, 9.08, 8.92 and 8.68 respectively on the $3^{rd}$ day, $7^{th}$ day, $14^{th}$ day, $21^{st}$ day and $28^{th}$ day. In resorbed teeth pH was 11.04, 11.38, 8.96, 8.58 and 8.48 respectively on the $3^{rd}$ day, $7^{th}$ day, $14^{th}$ day, $21^{st}$ day and $28^{th}$ day.

**Graph 6** shows the Change in pH During Retreatment in Intact and Resorbed Teeth with MTA Fillapex as Sealer. pH of intact teeth was 9.90, 10.98, 9.06, 8.8 and 8.56 respectively on the $3^{rd}$ day, $7^{th}$ day, $14^{th}$ day, $21^{st}$ day and $28^{th}$ day. In resorbed teeth pH was 10.82, 11.12, 8.86, 8.56 and 8.42 respectively on the $3^{rd}$ day, $7^{th}$ day, $14^{th}$ day, $21^{st}$ day and $28^{th}$ day.

**Graph 7** shows the Change in pH During Retreatment in Intact and Resorbed Teeth with BioRoot RCS as Sealer. pH of intact teeth was 9.78, 10.96, 8.86, 8.46 and 8.42 respectively on the 3rd day, 7th day, 14th day, 21st day and 28th day. In resorbed teeth pH was 10.68, 11.06, 8.78, 8.45 and 8.34 respectively on the 3rd day, 7th day, 14th day, 21st day and 28th day.

**Table 6** shows the Statistical Comparison of pH Changes During Retreatment in Intact Teeth. pH changes in intact teeth showed significance between the sealer groups at all intervals ($p<0.05$) except on 7th day($p=0.495$).

**Table 7** shows the Statistical Comparison of pH Changes During Retreatment in Intact Teeth on 3rd Day. There was significant difference between groups A and C; A and D; B and C; B and D; C and D (p value < 0.05) whereas no statistical significance was present between Groups - A and B ($p>0.05$).

**Table 8** shows the Statistical comparison of pH changes During Retreatment in Intact Teeth on 7th Day. There was no significant difference between Groups A and B; A and C; A and D; B and C; B and D; C and D (p = 0.827; 0.381; 0.214; 0.439; 0.212 and 0.729 respectively)

**Table 9** shows the Statistical comparison of pH Changes During Retreatment in Intact Teeth on 14th Day. There was significant difference between all the sealer groups A and B; A and C; A and D; B and D; C and D (p value < 0.05) whereas, there was no significant difference between Groups B and C (p value = 0.513).

**Table 10** shows the Statistical Comparison of pH Changes During Retreatment in Intact Teeth on 21st Day. There was no significant difference between Groups - A and B (p value = 0.221). Whereas, there was significance between groups A and C; A and D; B and C; B and D; C and D (p value = 0.013, 0.007, 0.018, 0.006 and 0.007 respectively).

**Table 11** shows the Statistical Comparison of pH Changes During Retreatment in Intact Teeth on 28th Day. There was significant difference between groups A and C; A and D; B and C; B and D; C and D (p value < 0.05) Whereas, there was no significant difference between groups A and B (p=0.18).

**Table 12** shows the Statistical Comparison of pH Changes During Retreatment in Resorbed Teeth. Resorbed teeth show significance between all groups at all intervals (p<0.05) except on 21st day (p=0.152).

**Table 13** shows the Statistical Comparison of pH Changes During Retreatment in Resorbed Teeth on 3rd Day. There was significant difference between groups A and C; A and D; B and C; B and D; C and D (p < 0.05) whereas, there was no significant difference between Groups A and B (p>0.05).

**Table 14** shows the Statistical comparison of pH changes During Retreatment in Resorbed Teeth on 7th Day. There was significant difference between Groups A and B; A and C; A and D; B and C; B and D (p value <

0.05), whereas, there was no significant difference between groups C and D ($p > 0.05$).

**Table 15** shows the Statistical comparison of pH Changes During Retreatment in Resorbed Teeth on 14th Day. There was significant difference between groups A and C; A and D; B and C; B and D ($p < 0.05$) whereas, there was no significant difference between Groups A and B; C and D ($p >0.05$).

**Table 16** shows the Statistical Comparison of pH Changes During Retreatment in Resorbed Teeth on 21st Day. There was significant difference between Groups A and D ($p = 0.031$). Whereas, there was no significance between groups A and B; A and C; B and C; B and D; C and D ($p > 0.05$).

**Table 17** shows the Statistical Comparison of pH Changes During Retreatment in Resorbed Teeth on 28th Day. There was significant difference between groups A and B; A and C; A and D; B and D; C and D ($p < 0.05$), whereas, there was no significant difference between groups B and C ($p=0.72$).

**Table 18** shows the Mean pH Change During Primary Endodontic Treatment and Retreatment in Intact Teeth. The mean difference in pH during primary endodontic treatment and during retreatment of Group A was - 1.5, -1, -1, -0.9 and -0.6 on the 3rd, 7th,14th, 21st and 28th day respectively. In Group B the difference was - 1.5 on the 3rd day decreasing by -1.02 on 7th day, -1.1 and

14$^{th}$ day, -0.9 on 21$^{st}$ day and -0.7 on the 28$^{th}$ day. In Group C the difference was -0.6 on the 3$^{rd}$ day, -1.1 on 7$^{th}$ day, -1.1 on 14$^{th}$ day, -1 on 21st day and -0.8 on the 28th day. In Group D the difference was - 1.8 on the 3$^{rd}$ day -1.1 on 7$^{th}$ day, -1.3 on 14$^{th}$ day, -1.4 on 21$^{st}$ day and -0.9 on the 28$^{th}$ day.

**Table 19** shows the Mean pH Change During Primary Endodontic Treatment and Retreatment in Resorbed Teeth. The difference in mean pH in Group A was -0.5, -0.8, -1.1, -1 and -0.6 on the 3$^{rd}$,7$^{th}$,14$^{th}$,21$^{st}$ and 28$^{th}$ day respectively. In Group B the difference was -0.6 on the 3$^{rd}$ day, -1 on 7$^{th}$ day, -1.2 0n 14$^{th}$ day, -1.1 on 21$^{st}$ day and -0.8 on 28th day. In Group C the difference was -0.7 on the 3$^{rd}$ day, -1.2 on 7$^{th}$ day, -1.3 on 14$^{th}$ day, -1.1 on 21$^{st}$ day and -0.8 on 28$^{th}$ day. In Group D the difference is -1 on the 3$^{rd}$ day, -1.3 on 7$^{th}$ day, -1.4 on 14$^{th}$ day, -1.2 on 21$^{st}$ day and -0.9 on 28$^{th}$ day.

**Table 20 and Graph 8** shows the Mean & S.D. pH Changes During Retreatment in Intact and Resorbed Teeth Irrespective of The Sealer Used. The mean pH values on 3$^{rd}$,7$^{th}$,14$^{th}$,21$^{st}$ and 28$^{th}$ day in Group 1 (intact teeth) were 9.940(±0.1314), 11.000(±0.0858), 9.040(±0.1231), 8.785(± 0.2084) and 8.595(± 0.1276) respectively, whereas in Group 2 (resorbed teeth) pH values were 10.925 (± 0.2099), 11. 270 (±0.2080), 8.900 (± 0.1076), 8. 580 (± 0.0768) and 8.465 (± 0.1182) on 3$^{rd}$,7$^{th}$,14$^{th}$,21$^{st}$ and 28$^{th}$ day respectively.

**Table 21** shows the Statistical Significance of pH change Between Intact and Resorbed Teeth During retreatment. There was statistical significance between Group 1 and Group 2 during retreatment ($p<0.05$) at all time periods.

**Table 22** shows the Statistical Significance of pH Change at $3^{rd}$, $7^{th}$, $14^{th}$, $21^{st}$ and $28^{th}$ day During Retreatment. There was significant difference ($p=0.000$) in pH at all time periods, in both intact and resorbed teeth. The mean pH on $3^{rd}$ and $7^{th}$ day reveals, resorbed teeth (Group 2) showed more mean pH level values than intact teeth (Group 1). But on the $14^{th}$, $21^{st}$ and $28^{th}$ day the pH mean values were marginally greater in intact teeth than resorbed teeth.

**Table 23** shows the Statistical Comparison of pH Changes During Primary Endodontic Treatment and Retreatment in Intact Teeth. Intra group comparison during retreatment shows significant difference in pH ($p=0.000$) at all time periods in intact teeth.

**Graph 9** shows the Statistical Comparison of pH Changes During Primary Endodontic Treatment and Retreatment in Intact Teeth. The mean pH during primary endodontic treatment and during retreatment showed that at all time periods the pH was higher during primary endodontic treatment than during retreatment

**Table 24** shows the Statistical Comparison of pH Changes During Primary Endodontic Treatment and Retreatment in Resorbed Teeth. Intra group comparison during retreatment shows significant difference in pH ($p<0.05$) at all time periods in resorbed teeth.

**Graph 10** shows the Comparison of pH Changes During Primary Endodontic Treatment and Retreatment in Resorbed Teeth. The mean pH changes during primary endodontic treatment and during retreatment showed that at all time periods the pH was higher during primary endodontic treatment than during retreatment in resorbed teeth.

# *Tables and Graphs*

## TABLES AND GRAPHS

### TABLE 1: MEAN ± S.D. pH CHANGES IN INTACT AND RESORBED TEETH DURING PRIMARY ENDODONTIC TREATMENT.

| | **Intact teeth** | **Resorbed teeth** |
|---|---|---|
| *3 days* | 11.505 ± 0.0945 | 11.625 ± 0.0786 |
| *7 days* | 12.04 ± 0.1046 | 12.345 ± 0.1191 |
| *14 days* | 10.195 ± 0.0826 | 10.125 ± .0716 |
| *21 days* | 9.825 ± 0.091 | 9.615 ± 0.0745 |
| *28 days* | 9.395 ± 0.0887 | 9.250 ± 0.0513 |

### GRAPH 1: MEAN pH CHANGES IN INTACT AND RESORBED TEETH DURING PRIMARY ENDODONTIC TREATMENT.

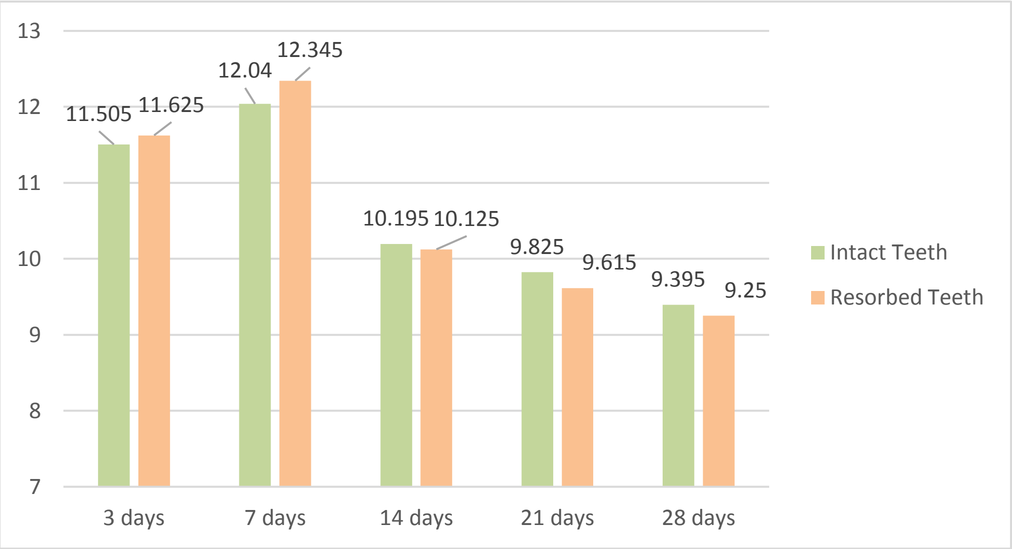


**TABLE 2: STATISTICAL COMPARISON OF pH CHANGES IN INTACT AND RESORBED TEETH DURING PRIMARY ENDODONTIC TREATMENT ON 3RD, 7TH, 14TH, 21ST AND 28TH DAY. #**

| | Group | Mean Rank | P value |
|---|---|---|---|
| 3 days | Intact teeth | 14.18 | .000* |
| | Resorbed teeth | 26.83 | |
| 7 days | Intact teeth | 11.23 | .000* |
| | Resorbed teeth | 29.78 | |
| 14 days | Intact teeth | 24.73 | .015* |
| | Resorbed teeth | 16.28 | |
| 21 days | Intact teeth | 29.80 | .000* |
| | Resorbed teeth | 11.20 | |
| 28 days | Intact teeth | 28.50 | .000* |
| | Resorbed teeth | 12.50 | |

**# Mann- Whitney Test, * Statistically significant**

**TABLE 3: STATISTICAL COMPARISON OF pH CHANGE ON 3RD, 7TH, 14TH, 21ST AND 28TH DAY DURING PRIMARY ENDODONTIC TREATMENT IN INTACT TEETH AND RESORBED TEETH##**

| | Days | P value |
|---|---|---|
| Intact teeth | 3 days | .000* |
| | 7 days | |
| | 14 days | |
| | 21 days | |
| | 28 days | |
| Resorbed teeth | 3 days | .000* |
| | 7 days | |
| | 14 days | |
| | 21 days | |
| | 28 days | |

**##Friedman Test, * Statistically significant**

**TABLE 4: MEAN & S.D. pH CHANGES IN INTACT TEETH DURING RETREATMENT ON 3RD, 7TH, 14TH, 21ST AND 28TH DAY.**

| | Group A | Group B | Group C | Group D |
|---|---|---|---|---|
| *3 days* | 10.060 ± 0.0548 | 10.020 ± 0.0837 | 9.900 ± 0.0707 | 9.780 ± 0.0837 |
| *7 days* | 11.040 ± 0.1140 | 11.020 ± 0.0837 | 10.980 ± 0.0837 | 10.960 ± 0.0548 |
| *14 days* | 9.160 ± 0.0548 | 9.080 ± 0.0447 | 9.060 ± 0.0548 | 8.860 ± 0.0548 |
| *21 days* | 8.960 ± 0.0548 | 8.920 ± 0.0447 | 8.800 ± 0.0707 | 8.460 ± 0.0548 |
| *28 days* | 8.720 ± 0.0447 | 8.680 ± 0.0447 | 8.560 ± 0.0548 | 8.420 ± 0.0447 |

**GRAPH 2: MEAN pH CHANGES IN INTACT TEETH DURING RETREATMENT ON 3RD, 7TH, 14TH, 21ST AND 28TH DAY.**

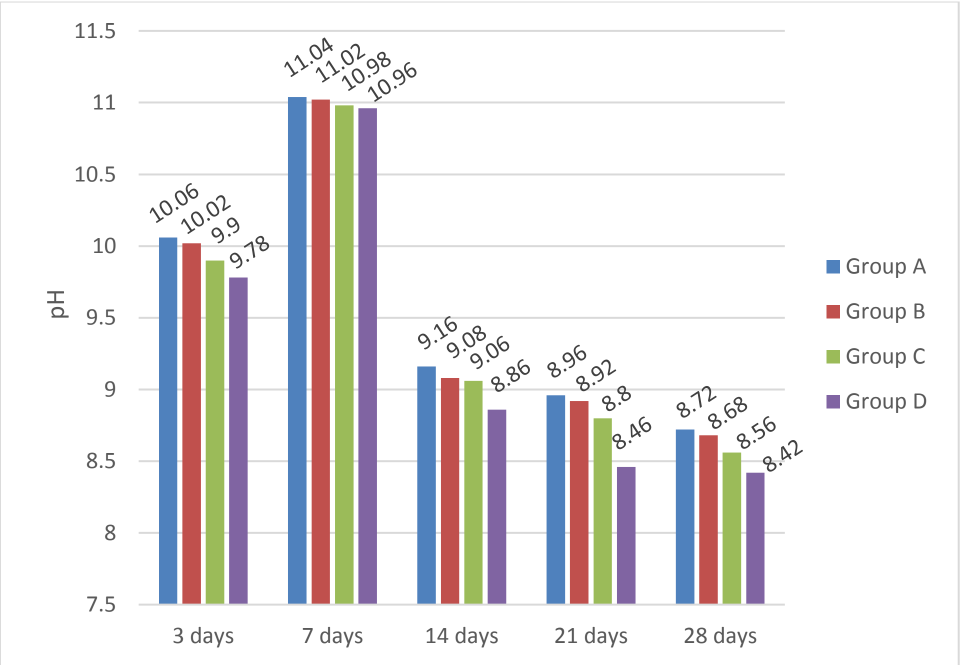
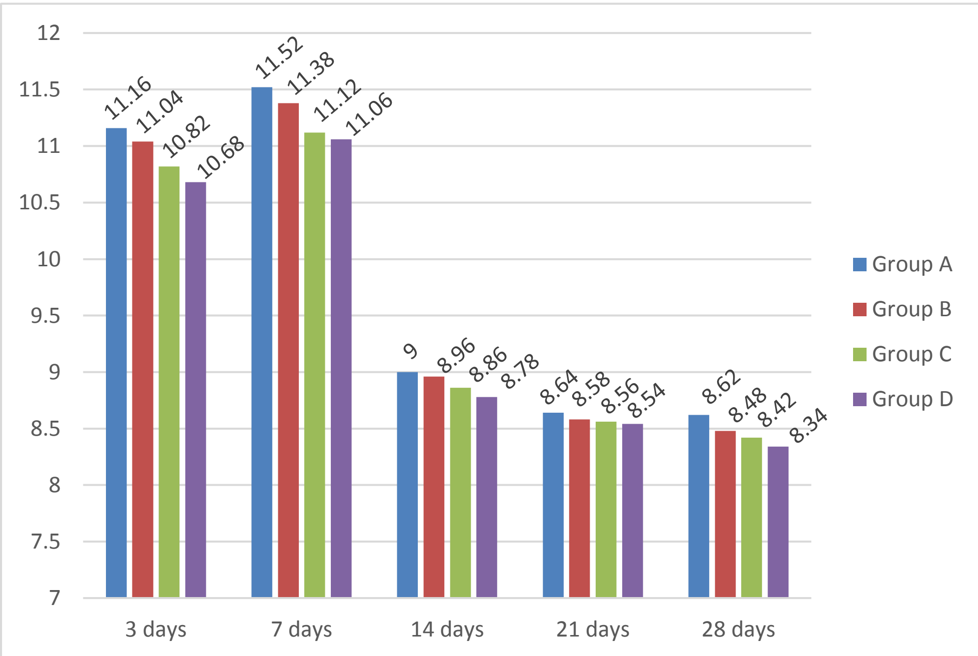


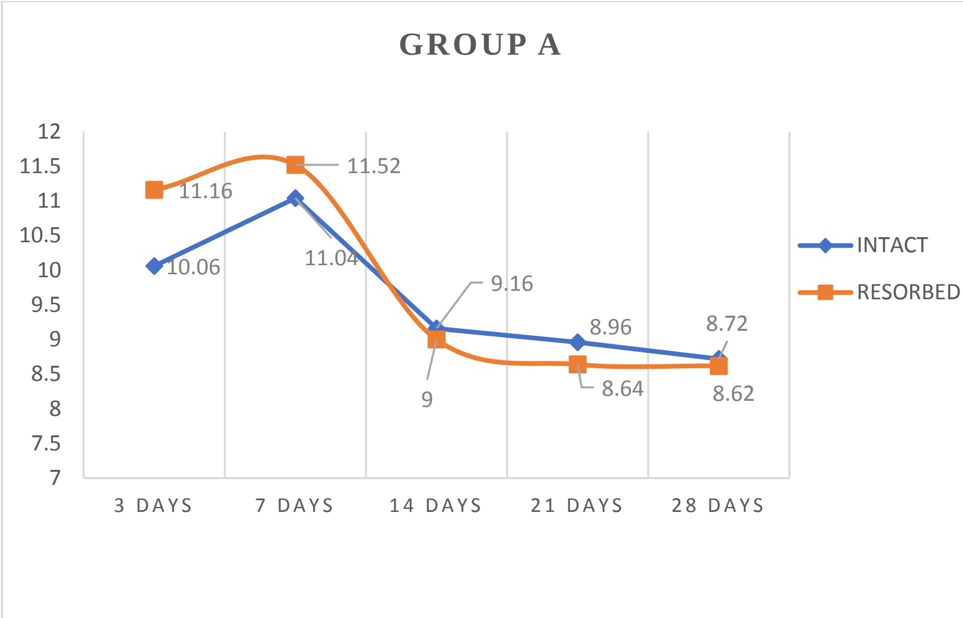
**TABLE 5: MEAN ± S.D. pH CHANGES DURING RETREATMENT IN RESORBED TEETH.**

| | GROUP A | GROUP B | GROUP C | GROUP D |
|---|---|---|---|---|
| *3 days* | 11.160 ± 0.1342 | 11.040 ± 0.0548 | 10.820 ± .0837 | 10.680 ± 0.0837 |
| *7 days* | 11.520 ± 0.1095 | 11.380 ± 0.0447 | 11.120 ± .0837 | 11.060 ± 0.0894 |
| *14 days* | 9.000 ± 0.0707 | 8.960 ± 0.0548 | 8.860 ± .0548 | 8.780 ± 0.0837 |
| *21 days* | 8.640 ± 0.0548 | 8.580 ± 0.0447 | 8.560 ± .1140 | 8.450 ± 0.0548 |
| *28 days* | 8.620 ± 0.0837 | 8.480 ± 0.0447 | 8.420 ± .0447 | 8.340 ± 0.0548 |

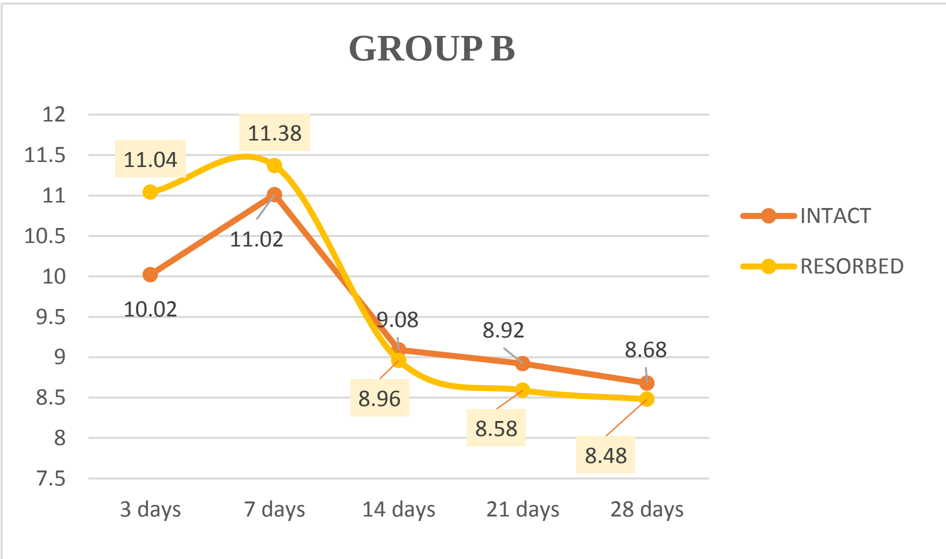
**GRAPH 3: MEAN pH CHANGES DURING RETREATMENT IN RESORBED TEETH.**



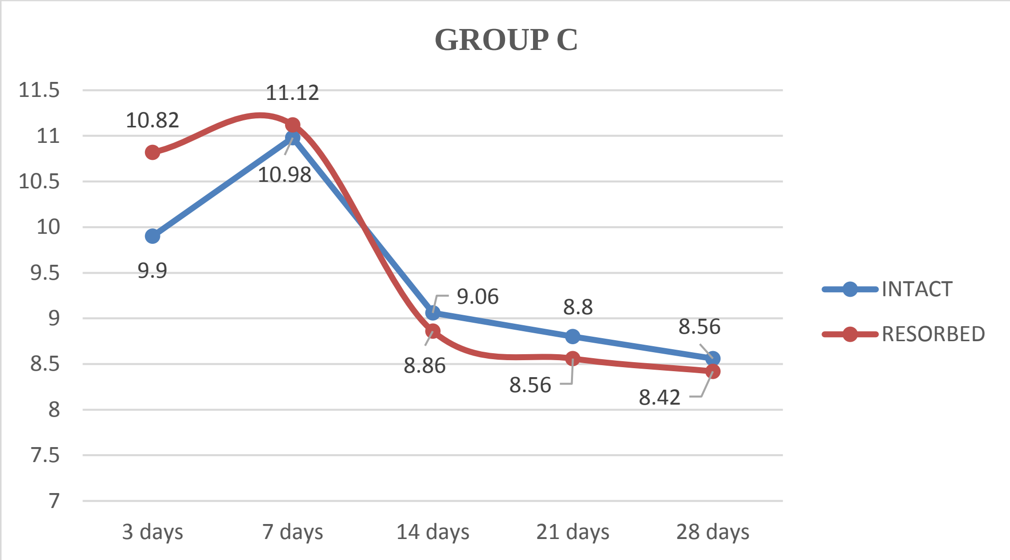
**GRAPH 4: CHANGE IN pH DURING RETREATMENT IN INTACT AND RESORBED TEETH WITH ZnOE AS SEALER.**



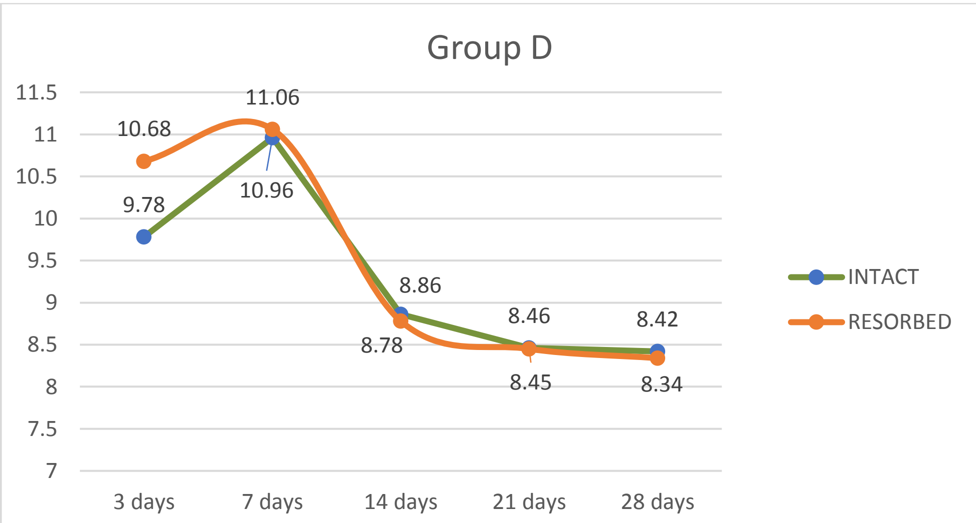

**GRAPH 5: CHANGE IN pH DURING RETREATMENT IN INTACT AND RESORBED TEETH WITH SEALAPEX AS SEALER**



**GRAPH 6: CHANGE IN pH DURING RETREATMENT IN INTACT AND RESORBED TEETH WITH MTA FILLAPEX AS SEALER**




**GRAPH 7: CHANGE IN pH DURING RETREATMENT IN INTACT AND RESORBED TEETH WITH BIOROOT RCS AS SEALER**



## TABLE 6: STATISTICAL COMPARISON OF pH CHANGES DURING RETREATMENT IN INTACT TEETH[###]

| | Group | Mean Rank | P value |
|---|---|---|---|
| *3 days* | Group A | 12.80 | .003* |
| | Group B | 18.00 | |
| | Group C | 7.50 | |
| | Group D | 3.70 | |
| *7 days* | Group A | 9.90 | .495 |
| | Group B | 18.00 | |
| | Group C | 7.50 | |
| | Group D | 6.60 | |
| *14 days* | Group A | 12.40 | .002* |
| | Group B | 18.00 | |
| | Group C | 8.60 | |
| | Group D | 3.00 | |
| *21 days* | Group A | 14.00 | .001* |
| | Group B | 16.80 | |
| | Group C | 8.20 | |
| | Group D | 3.00 | |
| *28 days* | Group A | 13.00 | .001* |
| | Group B | 18.00 | |
| | Group C | 7.80 | |
| | Group D | 3.20 | |

**[###] Kruskal-Wallis Test, * Statistically significant**

## TABLE 7: STATISTICAL COMPARISON OF pH CHANGES DURING RETREATMENT IN INTACT TEETH ON 3rd DAY[#]

| | Group | P value |
|---|---|---|
| 3 days | Group A | .419 |
| | Group B | |
| | Group A | .013* |
| | Group C | |
| | Group A | .008* |
| | Group D | |
| | Group B | .049* |
| | Group C | |
| | Group B | .011* |
| | Group D | |
| | Group C | .049* |
| | Group D | |

**[#] Mann- Whitney Test, * Statistically significant**

**TABLE 8: STATISTICAL COMPARISON OF pH CHANGES DURING RETREATMENT IN INTACT TEETH ON 7$^{TH}$ DAY. #**

| | Group | P value |
|---|---|---|
| 7 days | Group A | .827 |
| | Group B | |
| | Group A | .381 |
| | Group C | |
| | Group A | .214 |
| | Group D | |
| | Group B | .439 |
| | Group C | |
| | Group B | .212 |
| | Group D | |
| | Group C | .729 |
| | Group D | |

**# Mann- Whitney Test.**

**TABLE 9: STATISTICAL COMPARISON OF pH CHANGES DURING RETREATMENT IN INTACT TEETH ON 14$^{TH}$ DAY #**

| | Group | P value |
|---|---|---|
| 14 days | Group A | .042* |
| | Group B | |
| | Group A | .031* |
| | Group C | |
| | Group A | .007* |
| | Group D | |
| | Group B | .513 |
| | Group C | |
| | Group B | .006* |
| | Group D | |
| | Group C | .007* |
| | Group D | |

**# Mann- Whitney Test, * Statistically significant**

**TABLE 10: STATISTICAL COMPARISON OF pH CHANGES DURING RETREATMENT IN INTACT TEETH ON 21ST DAY #**

| | Group | P value |
|---|---|---|
| 21 days | Group A | .221 |
| | Group B | |
| | Group A | .013* |
| | Group C | |
| | Group A | .007* |
| | Group D | |
| | Group B | .018* |
| | Group C | |
| | Group B | .006* |
| | Group D | |
| | Group C | .007* |
| | Group D | |

**# Mann- Whitney Test, * Statistically significant**

**TABLE 11: STATISTICAL COMPARISON OF pH CHANGES DURING RETREATMENT IN INTACT TEETH ON 28TH DAY #**

| | Group | P value |
|---|---|---|
| 28 days | Group A | .180 |
| | Group B | |
| | Group A | .006* |
| | Group C | |
| | Group A | .005* |
| | Group D | |
| | Group B | .014* |
| | Group C | |
| | Group B | .005* |
| | Group D | |
| | Group C | .011* |
| | Group D | |

**# Mann- Whitney Test, * Statistically significant**

**TABLE 12: STATISTICAL COMPARISON OF pH CHANGES DURING RETREATMENT IN RESORBED TEETH###**

| | Group | Mean Rank | P value |
|---|---|---|---|
| *3 days* | Group A | 14.60 | .001* |
| | Group B | 16.40 | |
| | Group C | 7.40 | |
| | Group D | 3.60 | |
| *7 days* | Group A | 14.40 | .001* |
| | Group B | 16.60 | |
| | Group C | 6.50 | |
| | Group D | 4.50 | |
| *14 days* | Group A | 13.90 | .004* |
| | Group B | 16.70 | |
| | Group C | 7.20 | |
| | Group D | 4.20 | |
| *21 days* | Group A | 13.10 | .152 |
| | Group B | 13.70 | |
| | Group C | 8.60 | |
| | Group D | 6.60 | |
| *28 days* | Group A | 14.10 | .002* |
| | Group B | 16.80 | |
| | Group C | 7.30 | |
| | Group D | 3.80 | |

**### Kruskal-Wallis Test, * Statistically significant**

**TABLE 13: STATISTICAL COMPARISON OF pH CHANGES DURING RETREATMENT IN RESORBED TEETH ON 3RD DAY#**

| | Group | P value |
|---|---|---|
| 3 days | Group A | .118 |
| | Group B | |
| | Group A | .008* |
| | Group C | |
| | Group A | .008* |
| | Group D | |
| | Group B | .008* |
| | Group C | |
| | Group B | .008* |
| | Group D | |
| | Group C | .041* |
| | Group D | |

**# Mann- Whitney Test, * Statistically significant**

**TABLE 14: STATISTICAL COMPARISON OF pH CHANGES DURING RETREATMENT IN RESORBED TEETH ON 7TH DAY#**

| | Group | P value |
|---|---|---|
| 7 days | Group A | .018* |
| | Group B | |
| | Group A | .008* |
| | Group C | |
| | Group A | .007* |
| | Group D | |
| | Group B | .007* |
| | Group C | |
| | Group B | .006* |
| | Group D | |
| | Group C | .268 |
| | Group D | |

**# Mann- Whitney Test, * Statistically significant**

**TABLE 15: STATISTICAL COMPARISON OF pH CHANGES DURING RETREATMENT IN RESORBED TEETH ON 14TH DAY#**

| | Group | P value |
|---|---|---|
| 14 days | Group A | .339 |
| | Group B | |
| | Group A | .016* |
| | Group C | |
| | Group A | .010* |
| | Group D | |
| | Group B | .031* |
| | Group C | |
| | Group B | .013* |
| | Group D | |
| | Group C | .118 |
| | Group D | |

**# Mann- Whitney Test, * Statistically significant**

**TABLE 16: STATISTICAL COMPARISON OF pH CHANGES DURING RETREATMENT IN RESORBED TEETH ON 21$^{ST}$ DAY$^{\#}$**

| | Group | P value |
|---|---|---|
| 21 days | Group A | .093 |
| | Group B | |
| | Group A | .214 |
| | Group C | |
| | Group A | .031* |
| | Group D | |
| | Group B | .813 |
| | Group C | |
| | Group B | .221 |
| | Group D | |
| | Group C | .656 |
| | Group D | |

**# Mann- Whitney Test, * Statistically significant**

**TABLE 17: STATISTICAL COMPARISON OF pH CHANGES DURING RETREATMENT IN RESORBED TEETH ON 28$^{TH}$ DAY$^{\#}$**

| | Group | P value |
|---|---|---|
| 28 days | Group A | .018* |
| | Group B | |
| | Group A | .009* |
| | Group C | |
| | Group A | .008* |
| | Group D | |
| | Group B | .072 |
| | Group C | |
| | Group B | .011* |
| | Group D | |
| | Group C | .042* |
| | Group D | |

**# Mann- Whitney Test, * Statistically significant**

**TABLE 18: MEAN pH CHANGE DURING PRIMARY ENDODONTIC TREATMENT AND RETREATMENT IN INTACT TEETH**

| | | $3^{RD}$ DAY | $7^{TH}$ DAY | $14^{TH}$ DAY | $21^{ST}$ DAY | $28^{TH}$ DAY |
|---|---|---|---|---|---|---|
| **pH DURING TREATMENT** | | 11.505 | 12.04 | 10.195 | 9.825 | 9.395 |
| ***pH DURING RETREATMENT*** | ***ZnOE*** | 10.060(-1.5) | 11.040(-1) | 9.160(-0.9) | 8.960(-0.6) | 8.720(-0.6) |
| | ***Sealapex*** | 10.020(-1.5) | 11.020(-1) | 9.080(-1.1) | 8.920(-0.9) | 8.680(-0.7) |
| | ***MTA Fillapex*** | 9.900(-1.6) | 10.980(-1.1) | 9.060(-1.1) | 8.800(-1) | 8.560(-0.8) |
| | ***BioRoot RCS*** | 9.780(-1.8) | 10.960(-1.1) | 8.860(-1.3) | 8.460(-1.4) | 8.420(-0.9) |

**Within parentheses denotes difference in pH**

**TABLE 19: MEAN pH CHANGE DURING PRIMARY ENDODONTIC TREATMENT AND RETREATMENT IN RESORBED TEETH.**

| | | $3^{RD}$ DAY | $7^{TH}$ DAY | $14^{TH}$ DAY | $21^{ST}$ DAY | $28^{TH}$ DAY |
|---|---|---|---|---|---|---|
| **pH DURING TREATMENT** | | 11.625 | 12.345 | 10.125 | 9.615 | 9.250 |
| ***pH DURING RETREATMENT*** | ***ZnOE*** | 11.160(-0.5) | 11.520(-0.8) | 9.000(-1.1) | 8.640(-1) | 8.620(-0.6) |
| | ***Sealapex*** | 11.040(-0.6) | 11.380(-1) | 8.960(-1.2) | 8.580(-1.1) | 8.480(-0.8) |
| | ***MTA Fillapex*** | 10.820(-0.7) | 11.120(-1.2) | 8.860(-1.3) | 8.560(-1.1) | 8.420(-0.8) |
| | ***BioRoot RCS*** | 10.680(-1) | 11.060(-1.3) | 8.780(-1.4) | 8.450(-1.2) | 8.340(-0.9) |

**Within parentheses denotes difference in pH**

**TABLE 20: MEAN ± S.D. pH CHANGES DURING RETREATMENT IN INTACT AND RESORBED TEETH IRRESPECTIVE OF THE SEALER USED.**

| | Intact teeth | Resorbed teeth |
|---|---|---|
| *3 days* | 9.940 ± 0.1314 | 10.925 ± 0.2099 |
| *7 days* | 11.000 ± 0.0858 | 11. 270 ± 0.2080 |
| *14 days* | 9.040 ± 0.1231 | 8.900 ± 0.1076 |
| *21 days* | 8.785 ± 0.2084 | 8. 580 ± 0.0768 |
| *28 days* | 8.595 ± 0.1276 | 8.465 ± 0.1182 |

**GRAPH 8: MEAN pH CHANGES DURING RETREATMENT IN INTACT AND RESORBED TEETH IRRESPECTIVE OF THE SEALER USED.**

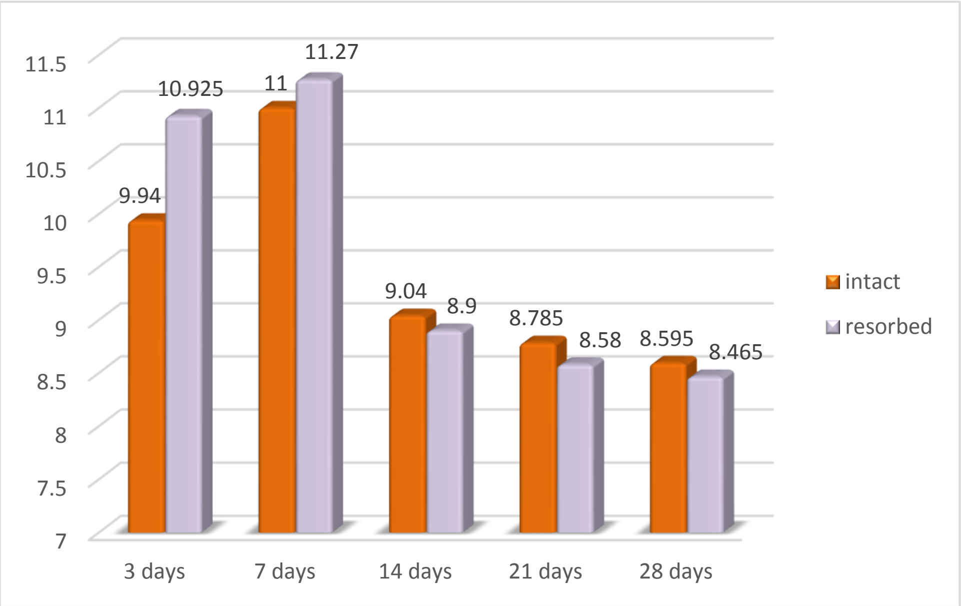


**TABLE 21: STATISTICAL SIGNIFICANCE OF pH CHANGE IN INTACT AND RESORBED TEETH DURING RETREATMENT. #**

| | Group | Mean Rank | P value |
|---|---|---|---|
| 3 days | Intact teeth | 10.50 | .000* |
| | Resorbed teeth | 30.50 | |
| 7 days | Intact teeth | 12.93 | .000* |
| | Resorbed teeth | 28.08 | |
| 14 days | Intact teeth | 26.50 | .001* |
| | Resorbed teeth | 14.50 | |
| 21 days | Intact teeth | 26.00 | .002* |
| | Resorbed teeth | 15.00 | |
| 28 days | Intact teeth | 25.75 | .004* |
| | Resorbed teeth | 15.25 | |

**# Mann- Whitney Test, * Statistically significant**

**TABLE 22: STATISTICAL SIGNIFICANCE OF pH CHANGE ON 3RD, 7TH, 14TH, 21ST AND 28TH DAY DURING RETREATMENT. ##**

| | Days | P value |
|---|---|---|
| Intact teeth | 3 days | .000* |
| | 7 days | |
| | 14 days | |
| | 21 days | |
| | 28 days | |
| Resorbed teeth | 3 days | .000* |
| | 7 days | |
| | 14 days | |
| | 21 days | |
| | 28 days | |

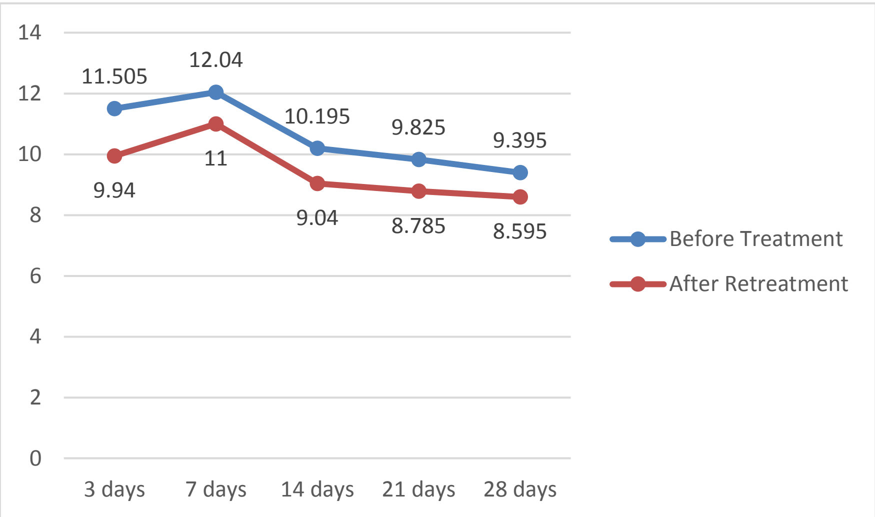
**##Friedman Test, * Statistically significant**

**TABLE 23: STATISTICAL COMPARISON OF pH CHANGES DURING PRIMARY ENDODONTIC TREATMENT AND RETREATMENT IN INTACT TEETH [#]**

| | Group | Mean | SD | Mean Rank | P value |
|---|---|---|---|---|---|
| **3 days** | During treatment | 11.505 | .0945 | 30.5 | .000* |
| | During retreatment | 9.940 | .1314 | 10.5 | |
| **7 days** | During treatment | 12.040 | .1046 | 30.5 | .000* |
| | During retreatment | 11.000 | .0858 | 10.5 | |
| **14 days** | During treatment | 10.195 | .0826 | 30.5 | .000* |
| | During retreatment | 9.040 | .1231 | 10.5 | |
| **21 days** | During treatment | 9.825 | .0910 | 30.5 | .000* |
| | During retreatment | 8.785 | .2084 | 10.5 | |
| **28 days** | During treatment | 9.395 | .0887 | 30.5 | .000* |
| | During retreatment | 8.595 | .1276 | 10.5 | |

**[#] Mann- Whitney Test, * Statistically significant**

**GRAPH 9: STATISTICAL COMPARISON OF pH CHANGES DURING PRIMARY ENDODONTIC TREATMENT AND RETREATMENT IN INTACT TEETH**

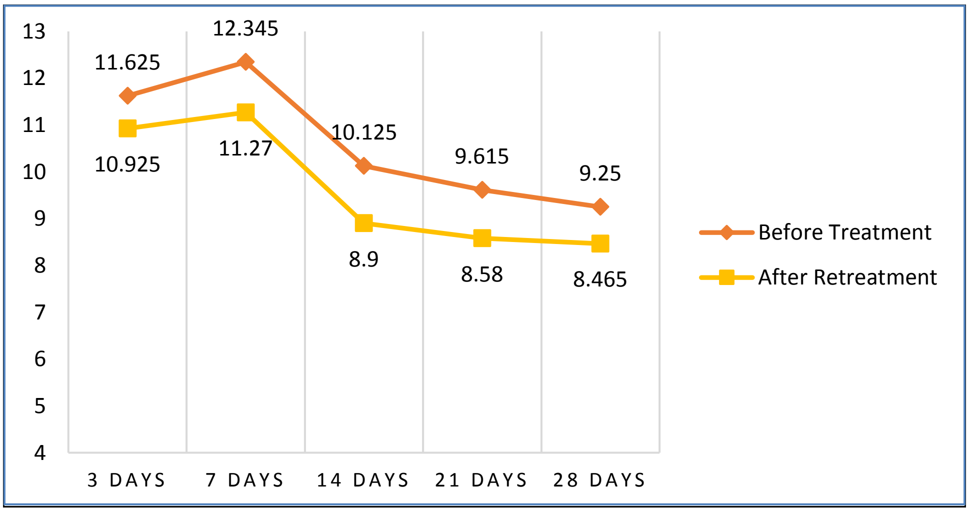


## TABLE 24: STATISTICAL COMPARISON OF pH CHANGES DURING PRIMARY ENDODONTIC TREATMENT AND RETREATMENT IN RESORBED TEETH#

| | Group | Mean | SD | Mean Rank | P value |
|---|---|---|---|---|---|
| **3 days** | During treatment | 11.625 | .0786 | 30.5 | .000* |
| | During retreatment | 10.925 | .2099 | 10.5 | |
| **7 days** | During treatment | 12.345 | .1191 | 30.5 | .000* |
| | During retreatment | 11.270 | .2080 | 10.5 | |
| **14 days** | During treatment | 10.125 | .0716 | 30.5 | .000* |
| | During retreatment | 8.900 | .1076 | 10.5 | |
| **21 days** | During treatment | 9.615 | .0745 | 30.5 | .000* |
| | During retreatment | 8.580 | .0768 | 10.5 | |
| **28 days** | During treatment | 9.250 | .0513 | 30.5 | .000* |
| | During retreatment | 8.465 | .1182 | 10.5 | |

**# Mann- Whitney Test, * Statistically significant**

## GRAPH 10: STATISTICAL COMPARISON OF pH CHANGES DURING PRIMARY ENDODONTIC TREATMENT AND RETREATMENT IN RESORBED TEETH



# *Discussion*

# DISCUSSION

Evidence based studies have proved that primary root canal treatment is a very reliable treatment with success rates ranging from 86% to 98%. However, failures can occur ensuing a need for clinical intervention. The two most common causes of endodontic failures are the presence of root canal complexities which has not been identified or inadequately disinfected, and failure of coronal seal leading to reentry of micro-organisms into the root canal system. This leads to harboring of microbial flora in the root canals resulting in secondary infection.

Nonsurgical retreatment aims at the removal of the existing root filling materials, followed by thorough disinfection of the entire root canal system and 3-dimensional obturation. It is generally considered the first treatment of choice in management of endodontically failed teeth showing a success rate of 78% to 80.4% (Torabinejad et al.).[49] Ng et al. reported that the success rate of nonsurgical retreatment showing complete healing was 77%.[31]

A difference between primary root canal treatment and re-treatment is the need to retrieve the existing root canal filling material and complete the disinfection. The most commonly used root canal filling material is gutta percha. Gutta-percha is generally used as a core filling material along with a root canal sealer of choice. Gutta-percha can be retrieved using hand, rotary or ultrasonic instruments with or without the help of heat or solvents such as chloroform, xylol, eucalyptol, halothane or essential oils. Most traditional endodontic sealers are zinc oxide–eugenol sealers and calcium hydroxide sealers. Later on, glass ionomer sealers, resin-based sealers were introduced and the newer sealers are MTA and Bioceramic sealers. Zinc oxide–eugenol sealers can be removed readily with mechanical instrumentation, with or without the aid of solvents, whereas other sealers are more difficult to remove as they are practically insoluble in solvents and are adherent to the root canal wall. [10]

Removing the maximum amount of filling material from inadequately prepared or filled root canal systems appears to be essential in order to enable re-instrumentation and thorough chemomechanical disinfection of the root canal system. Besides, the residual filling material may also interfere with the adhesion and adaptation of root filling materials in subsequent retreatment which may affect the rate of success.[19]

Currently, the use of rotary instruments is preferred over conventional hand instruments for the removal of root fillings due to their increased efficiency and the reduced time taken. The commonly available rotary retreatment instruments are protaper universal retreatment files, Mtwo-R and R endo files.[10] Takahashi et al (2009) proved that protaper universal rotary retreatment system without use of solvents was faster in removing the root filling materials.[45] Hulsmann and Bluhm (2004)[21] have reported that since Protaper instruments present a negative cutting angle, they might yield better results in terms of working length and root canal cleanliness than manual instruments. Studies have reported Protaper Universal retreatment files system is also more effective than the other rotary retreatment systems in the retreatment efficacy and time taken to retrieve the filling materials.[22] So, protaper universal retreatment system was used in this study.

Although one of the aims of re-treatment is to completely remove the previously placed root filling material, endodontic literature reveals complete clearance of the material is impossible. L.S. Gu et al (2008) stated that all techniques left GP/sealer remnants on root canal walls.[18]

Disinfection is the main issue when dealing with re-treatment cases. Calcium hydroxide is considered as the gold standard intracanal medicament and is still widely used after its introduction by Hermann in 1920. Calcium hydroxide is highly alkaline substance with a pH of approximately 12.5 – 12.8.[29] It dissociates into calcium and hydroxyl ions in an aqueous solution. The main actions are its antibacterial property and its ability to induce hard-tissue deposition (Siqueira & Lopes 1999)[41]. These hydroxyl ions alter the integrity of the bacterial cytoplasmic membrane as a result of the toxic effects generated during the transfer of nutrients or by destruction of the phospholipids of unsaturated fatty acids.[12] The maintenance of a high concentration of hydroxyl ions can change and inactivate the enzymatic activity.[13] Hence, most of the root canal pathogens cannot survive in the highly alkaline environment provided by calcium hydroxide.[41] In addition, use of calcium hydroxide paste in teeth with apical periodontitis has shown better healing of periapical tissues.[12]

The hydroxyl group encourages repair and active calcification due to its high alkalinity. This alkaline pH neutralizes lactic acid from osteoclasts, thus preventing dissolution of the minerals and also activates an enzyme, alkaline phosphatase, that play an important role in hard-tissue formation. The pH necessary for the activation of this enzyme varies from 8.6 to 10.3 (Estrela et al.). Alkaline phosphatase is a hydrolytic enzyme that acts by liberation of inorganic phosphatase from the phosphate esters, thus freeing the phosphate ions, which then reacts with the calcium ions from bloodstream to form calcium phosphate precipitate. This precipitate is the molecular unit of hydroxyapatite.[29] Literature shows that calcium hydroxide can arrest external root resorption and accelerate healing by diffusing through dentinal tubules and communicating with the periodontal ligament space.[44]

Studies show that an ideal intracanal medicament should penetrate deeply through the dentinal tubules for its antimicrobial activity and prevention of reinfection. The rate of diffusion of calcium hydroxide is dependent on several factors like buffering action of dentin, duration of medicament placed in the canals and the type of vehicle used with calcium hydroxide powder. Root dentine has a variable permeability for ionic diffusion depending on the diameter, number, direction of dentinal tubules and the thickness of the root canal walls.[33,46] The tubule penetration of intracanal medicament depends on its surface tension; low surface tension provides more penetration into the dentinal tubules. Viscous vehicles are water-soluble substances that release calcium and hydroxyl ions slowly for longer periods**.** A viscous vehicle may remain within root canal for several months, hence may reduce the number of appointments required to change the dressing.[29] Among aqueous based preparations of calcium hydroxide RC Cal performed better and can be preferred over calcium hydroxide points or oil-based calcium hydroxide preparations.[42] RC Cal pastes liberate significantly more calcium and hydroxyl ions and raise the pH higher than other calcium hydroxide preparations.[17] So, RC Cal was used in this study.

Dentine permeability is an essential factor for diffusion of hydroxyl ions. Smear layer formed during root canal instrumentation, blocks the entrances to lateral canals and dentinal tubules, thus reducing dentin permeability. Teixeira et al. (2005)[48] suggested the use of EDTA and sodium hypochlorite to remove the smear layer. NaOCl is an organic solvent, whereas EDTA has a slight dissolving effect on most root canal sealers (Keles 2009)[25] and dissolve the inorganic components of root dentin[15] making it easier to mechanically remove the root filling.[52] Hence the use of EDTA and sodium hypochlorite was advocated in this study.

In case of endodontic retreatments dentine permeability however may be reduced due to two reasons: (i) Calcium hydroxide medicament used during primary endodontic treatment, produces calcium phosphate by the interaction of ionized calcium with the phosphate ions of dentine, thus reducing dentin permeability.[33] (ii) Secondly, the obturation performed in the primary treatment also decreases dentine permeability due to the penetration of sealer into dentinal tubules and lateral canals.

Kouvas et al. (1998)[26], Ordinola-Zapata et al. (2009)[32] showed that the sealers can penetrate and reach different depths influenced by their physicochemical properties such as composition, film thickness and flow.

Tronstad et al, Tao et al, and Nerwich et al showed that the presence of root cementum could prevent or hinder ionic diffusion. Cementum is the calcified avascular mesenchymal tissue that forms the outer covering of the anatomic root. It contains 45-50 % Inorganic substances and 50-55% Organic substances. Regarding the permeability of cementum, it is shown that cementum is permeable from dentin towards the periodontal ligament side. Cellular cementum is more permeable than acellular cementum. Alyahya et al[1] showed that the absence of cementum facilitated bacterial penetration into the dentinal tubules. Inflammatory root resorption causes pockets of cementum loss (resorption) around the root, which may be due to physiologic or pathologic dissolution of the mineralized tissue.

Previous studies by Sherma Saif et al[38] and Roghanizad et al[36] have compared the hydroxyl ion diffusion in both intact teeth and resorbed teeth. The presence of mineralized tissue such as dentin and cementum have a role on the permeability of hydroxyl ion diffusion from the canal into surrounding tissues. This in turn would have a bearing on the pH of the surrounding tissues to maintain its alkalinity.

The diffusion of hydroxyl ions in intact and resorbed teeth were compared in this study. The null hypothesis was that the absence of cementum in resorbed areas of teeth do not cause change in pH.

In this study single rooted mandibular premolars were selected as they are the commonly extracted teeth during orthodontic treatment and show minimal variations with respect to number of canals, length and width. They have adequate wall thickness which can facilitate preparation of simulated resorptive defects. The teeth were decoronated to standardize the length of the root to 14 mm. The apical third of all the roots were covered by cold cure acrylic to standardize all the teeth as the apical third showed higher concentration of lateral canals. The apical end of the specimen was flattened to facilitate placement in the respective containers. This facilitated the exposure of cervical and middle thirds of the root to the permeability of intracanal medicament as the number of dentinal tubules are more in this region. The samples were divided into 2 groups of 20 teeth each. Group 1 was intact teeth and Group 2 was simulated resorbed teeth. In Group 2 wells were created on the external root surface to simulate root resorption, in accordance to Kazemipoor et al (2012)[24].

Primary endodontic treatment was initiated in all the specimens using protaper rotary instruments upto F3, and irrigation was done with 17% EDTA and 3% NaOCl. The canals were completely dried using paper points and intracanal medication of the canal walls was done with RC Cal. The access cavity was closed with IRM and sealed with varnish to prevent coronal leaching of calcium hydroxide. All the specimens were then suspended in a 30ml plastic container containing 10ml of deionized water as it is free of minerals and ions and has a pH of nearly 7. A digital pH meter was used to measure the pH on the $3^{rd}$,$7^{th}$ ,$14^{th}$ ,$21^{st}$ and $28^{th}$ day during the study.

The mean pH changes during primary endodontic treatment shows that in Group 1(intact teeth) due to the diffusion of hydroxyl ions into the deionized water, the initial pH of deionized water (pH-7) gradually increased to approximately 11.50 on the $3^{rd}$ day and reached a peak pH of approximately 12.04 on the $7^{th}$ day. Then, the pH reduced gradually from $14^{th}$ day reaching a pH of approximately 9.39 on the $28^{th}$ day. (Table 1; Graph 1) There was significant difference in pH changes at all time periods in Group 1 (Table 3). The rate of hydroxyl ion diffusion in the decreasing order was depicted as $7^{th}$ day > $3^{rd}$ day > $14^{th}$ day > $21^{st}$ day > $28^{th}$ day

In Group 2 (resorbed teeth) pH elevated faster for the $1^{st}$ three days raising the pH approximately to 11.6. By the end of one-week peak pH value of approximately 12.3 was obtained. By the end of day 14, the pH starts reducing to 10.12 and by day 28 reaches a pH of 9.25 (Table 1; Graph 1). There was significant difference in pH changes at all time periods in Group 2 (Table 3). The rate of hydroxyl ion diffusion in resorbed teeth was characterized as $7^{th}$ day > $3^{rd}$ day > $14^{th}$ day > $21^{st}$ day > $28^{th}$ day in the decreasing order.

Comparison between Group 1 and Group 2 shows that there was significant difference between the groups at all time periods (Table 2). The mean pH values show that the hydroxyl ion diffusion was more in resorbed teeth (Group 2) than the intact teeth (Group 1) in 3$^{rd}$ and 7$^{th}$ day, whereas, pH values on the 14$^{th}$,21$^{st}$ and 28$^{th}$ day showed that the diffusion of hydroxyl ion was higher in the intact teeth (Group 1) than resorbed teeth (Group 2).

The results of this study were supported by Sherma Saif (2008)[38] who stated that defects on the root surface disrupt the negative membrane potential of cementum thus allowing faster ion diffusion. Contradictory findings were reported by Roghanizad et al (2011) who showed that diffusion of hydroxyl ions was similar in both intact and resorbed roots. This may probably be due to the lesser size and number of wells made on the samples to simulate root resorption thereby indicating that there were reduced number of open dentinal tubules for hydroxyl ion penetration ensuing similar amount of diffusion between intact and resorbed teeth in their study.

In the present study, pH values on the 14$^{th}$,21$^{st}$ and 28$^{th}$ day was higher in the intact teeth (Group 1) than resorbed teeth (Group 2). This may be due to faster release of the calcium ions and hydroxyl ions into deionized water till 7 days, which may lead to a level of hyper or super saturation externally producing higher pH. This external super saturated solution then slowly tends to attain a status of dynamic equilibrium, as the walls of the intact teeth act like a semi-permeable membrane, with the internal solution resulting in lowering of pH with time (14$^{th}$ day>21$^{st}$ day >28$^{th}$ day). Rehman et al showed that the release of calcium ions into the deionized water was highest by the end of one week and gradually reduced in the subsequent weeks.[29] During this phase the calcium ions and hydroxyl ions re-diffuse into the canals slowly to attain an equilibrium state lowering the pH externally.

In resorbed teeth the excess calcium ions tend to form a precipitate on the irregular surfaces of resorbed teeth. This may probably result in higher concentration of hydroxyl ions externally, which may diffuse internally due to the concentration gradient resulting in a marginal lowering of the pH externally (in deionized water) compared to intact teeth (14$^{th}$ day>21$^{st}$ day >28$^{th}$ day).

Thus, in this study the first hypothesis was rejected as the absence of cementum in resorbed areas of teeth causes change in rate of hydroxyl ion diffusion.

The root samples were retrieved from their respective containers. Calcium hydroxide intracanal medicament was removed by copious irrigation with 3% sodium hypochlorite and 17% EDTA and ultrasonic activation was done using irrisafe ultrasonic tip. Final irrigation was done with saline. The samples were then placed in their respective containers replaced with a fresh deionized water and maintained for 7 days to ensure the neutralization of pH changes in root dentin following removal of calcium hydroxide medicament.

The samples in Group 1 and Group 2 were further subdivided into 4 groups each based on the sealers used for obturation namely Group A (Zinc oxide eugenol sealer), Group B (Sealapex), Group C (MTA fillapex) and Group

D (BioRoot RCS). Zinc oxide eugenol is a traditional sealer which is still in use. Sealapex is a non-eugenol calcium hydroxide based polymeric root canal sealer which promotes rapid healing and formation of hard tissue. MTA Fillapex is a salicylate resin cement containing silica nanoparticles, and 13.2% set mineral trioxide aggregate. MTA Fillapex has good sealer penetration and higher solubility values. MTA Fillapex and Sealapex are biocompatible and also stimulate mineralization. This is because the CaO present in MTA Fillapex and Sealapex, when in contact with water, gets converted into calcium hydroxide which further dissociates into calcium and hydroxyl ions. BioRoot RCS is a new bioactive tricalcium silicate water-based sealer. It has an ability to release calcium hydroxide after setting. This property was absent in MTA fillapex.[39]

The root canals were then dried with paper points and the teeth in each subgroup were obturated with the respective root canal sealers and 2% gutta-percha points by lateral condensation technique (considered gold standard). The samples were maintained for 7 days to allow complete setting of root canal sealers.

The root fillings (GP and Sealer) were then removed in all the samples using protaper universal retreatment files D1, D2 and D3 in crown down sequence. Each file has different length, taper, and apical tip diameter. The D1 instrument has an active tip to facilitate initial penetration into the coronal third of the filling material; The D2 instrument is for removal of filling material at the level of the middle third and D3 instrument in the apical third and used to reach the working length.[18] Followed by retrieval of filling materials the samples were copiously irrigated with 3% sodium hypochlorite and 17% EDTA.

Change in pH of dentine due to some endodontic sealers were noted by Tagger et al. (1998) and Torabinejad et al. (1995), so after removal of filling materials the teeth were stored for 7 days before placement of calcium hydroxide medicament.[9]

The canals were then completely dried using paper points and intracanal medication of the canal walls was done with RC Cal. The access cavity was closed with IRM and coated with varnish. All the specimens were then suspended in a fresh solution of 10ml deionized water. A digital pH meter was used to measure the pH of the samples on $3^{rd}$,$7^{th}$ ,$14^{th}$ ,$21^{st}$ and $28^{th}$ day of the study.

The mean pH during retreatment on $3^{rd}$,$7^{th}$ ,$14^{th}$ ,$21^{st}$ and $28^{th}$ day in Group 1- intact teeth (Table 4 and Graph 2) and Group 2 - resorbed teeth (Table 5 and Graph 3) revealed that at all time periods Group A (Zinc oxide eugenol sealer) showed highest pH followed by Group B (Sealapex), Group C (MTA fillapex) and Group D (BioRoot RCS). Regarding the time interval and rate of hydroxyl ion diffusion it was noted that greatest diffusion was present on $7^{th}$ day followed by $3^{rd}$ day, $14^{th}$ day, $21^{st}$ day and least diffusion was seen on $28^{th}$ day irrespective of the sealer groups.

In Group A (Zinc oxide eugenol sealer) the maximum hydroxyl ion diffusion in intact teeth was seen by the end of $1^{st}$ week showing approximately 11.04 pH followed by gradual decrease of pH in subsequent weeks [$14^{th}$ day (pH 9.16) > $21^{st}$ day (8.96) >$28^{th}$ day (8.72)]. In resorbed teeth for Group A the pH increased from 11.16 to 11.52 by the end of $1^{st}$ week followed by gradual decrease of pH in subsequent weeks [$14^{th}$ day (pH 9) > $21^{st}$ day (8.64) > $28^{th}$ day (8.62)] (Graph 4). Comparing intact and resorbed teeth in Group A, resorbed teeth had higher pH values in comparison to intact teeth till the end of $1^{st}$ week and later the intact teeth showed marginally higher pH than resorbed teeth.

In Group B (Sealapex) the maximum hydroxyl ion diffusion in intact teeth by the end of $1^{st}$ week was approximately 11.02 pH followed by gradual decrease of pH in subsequent weeks [$14^{th}$ day (pH 9.08) > $21^{st}$ day (8.92) > $28^{th}$ day (8.68)]. In resorbed teeth for Group B(Sealapex), the pH increased to 11.38 by the end of $1^{st}$ week followed by gradual decrease of pH in subsequent weeks [$14^{th}$ day (pH 8.96) > $21^{st}$ day (8.58) >$28^{th}$ day (8.48)] (Graph 5). Comparing intact and resorbed teeth in Group B, resorbed teeth had higher pH values in comparison to intact teeth till the end of $1^{st}$ week and later the intact teeth showed marginally higher pH than resorbed teeth.

Group C (MTA fillapex) showed a peak pH at the end of $1^{st}$ week was 10.98 followed by gradual decrease of pH in subsequent weeks [$14^{th}$ day (pH 9.06) > $21^{st}$ day (8.8) >$28^{th}$ day (8.56)]. In resorbed teeth for Group C (MTA fillapex), the peak pH was approximately 11.12 on 7th day and a lowest pH of approximately 8.42 was noted on day 28 [14th day (pH 8.86) > 21st day (8.56) > 28th day (8.42)] (Graph 6). Comparing intact and resorbed teeth in Group C, resorbed teeth had higher pH values in comparison to intact teeth till the end of 1st week and later the intact teeth showed higher pH than resorbed teeth.

Group D (BioRoot RCS) showed that pH increased to 10.96 from 9.78 by 7 days and then slowly decreased to a least value of 8.42 at the end of 28 day [14th day (pH 8.86) > 21st day (8.46) >28th day (8.42)]. In resorbed teeth, the greatest pH of approximately 11.06 on 7th day which gradually reduced to approximately 8.34 on 28th day [14th day (pH 8.78) > 21st day (8.45) > 28th day (8.34)] (Graph 7). Comparing intact and resorbed teeth in Group D, resorbed teeth had higher pH values in comparison to intact teeth till the end of 1st week and later the intact teeth showed marginally higher pH than resorbed teeth.

Comparison of pH changes of all the sealer groups (Group A-D) during retreatment in intact teeth showed that there was significant difference in the diffusion of hydroxyl ions between the groups on all intervals, except on 7th day where no significant difference was perceived (Table 6). Post HOC analysis showed that on the 3rd and 21st day there was significant difference between all the groups except Group A and Group B which showed no significant difference. On 14th and 28th day no significant difference was shown in Group B and Group C, whereas other groups showed significant difference (Tables 7-

11). This can be attributed to the diffusivity of hydroxyl ions from calcium hydroxide placed after the removal of different sealers.

In resorbed teeth, no significant difference was seen in the diffusion of hydroxyl ions between the sealer groups on 21$^{st}$ day whereas, there was significant difference on 3$^{rd}$,7$^{th}$,14$^{th}$ and 28$^{th}$ day (Table 12). Post HOC analysis showed that on the 3$^{rd}$ day there was significant difference between all the groups except Group A and Group B which showed no significant difference (Table 13). On the 7$^{th}$ day there was significant difference in all groups except Group C and Group D (Table 14). On the 14$^{th}$ day there was significant difference in all groups except between Group A and B; and Group C and D (Table 15). On day 21 except groups A and D all other groups showed no significant difference (Table 16). On 28$^{th}$ day no significant difference was shown between Group B and Group C, whereas other groups showed significant difference (Table 17). Thus, in this study it was shown that hydroxyl ion diffusion of calcium hydroxide is dependent on the type of sealer being used in retreatment cases and the amount of calcium hydroxide that was left in the dentinal tubules after removal during retreatment.

At all time intervals, in both intact and resorbed teeth it was seen that the diffusion of hydroxyl ions was greatest in Group A (Zinc oxide eugenol sealer) and least in Group D (BioRoot RCS). The decreasing order of groups according to rate of hydroxyl ion diffusion was Group A > Group B > Group C > Group D. This can be due to the flow rate of different sealers and the depth of sealer penetration into dentinal tubules which in turn is related to the physical and chemical properties of the sealers.

The Zinc oxide eugenol based sealers have weak sealing and adhesion to dentin.[3] The depth of penetration of the Zinc oxide eugenol based sealer has been shown to be less than 100 µm into the dentinal tubules whereas resin based sealers penetrated upto 751–1000 µm into dentin.[27] Eric et al [3] showed that Zinc oxide eugenol showed reasonable amount of dentinal penetration whereas calcium hydroxide based sealer showed good penetration into the dentinal tubules. Unlike resin based sealers, ZnOE-based sealer showed a granular appearance under scanning electron microscope.[27] Hence the consistency of root canal sealer can also affect the depth of penetration into dentinal tubules.

When comparing the pH change in intact teeth (Group A) during primary endodontic treatment and retreatment, there is a definite fall in pH on the 3$^{rd}$ day (- 1.5) decreasing by -1 on 7$^{th}$ day and 14$^{th}$ day, -0.9 on 21$^{st}$ day and -0.6 on the 28$^{th}$ day (Table 18). Initially the drop in pH may be attributed to the reason that Zinc oxide eugenol is not a hydroxyl ion dissociating sealer and the hydroxyl ions from the calcium hydroxide placed during retreatment diffuses less into the deionized water, attaining maximum diffusion by day 7 and tending to attain equilibrium by 28$^{th}$ day. In resorbed teeth, the change in pH is -0.5 on the 3$^{rd}$ day, -0.8 on 7$^{th}$ day, -1.1 0n 14$^{th}$ day, -1 on 21$^{st}$ day and -0.6 on 28$^{th}$ day (Table 19). This may be attributed to the partial absence of cemental barrier. Due to diffusion of calcium and hydroxyl ions the deionized water becomes supersaturated by14th day and tries to attain equilibrium by the 28$^{th}$ day. So, it can be concluded that the composition of the sealer (ZnOE) does not play a role in the diffusion of hydroxyl ions both during primary endodontic treatment and retreatment.

Sealapex (Group B) is a viscous mixture of calcium hydroxide and iodoform. It has a weak matrix and exhibits high water absorption. These properties of Sealapex permit a marked ingress of water facilitating a continuous reaction between the calcium powder and binder, leading to release of calcium and hydrogen ions which may induce formation of mineralization within the dentinal tubules.[44] Kouvas et al. 1998 and Ordinola-Zapata et al. 2009 showed that the greater intratubular penetration of Sealapex hindered the removal of sealer during retreatment and reduced the dentin permeability.

When comparing the pH change in intact teeth (Group B) during primary endodontic treatment and retreatment, there is a definite fall in pH on the 3$^{rd}$ day (- 1.5) decreasing by -1.02 on 7$^{th}$ day, -1.1 and 14$^{th}$ day, -0.9 on 21$^{st}$ day and -0.7 on the 28$^{th}$ day (Table 18). Initially the drop in pH may be attributed to the reason that Sealapex, a calcium hydroxide containing cement penetrates deep into the dentinal tubules and the calcium ions left within the dentinal tubules reduces the diffusivity of hydroxyl ions thereby initially reducing the pH by 3$^{rd}$ day, increasing by day 7, reducing by day 14 probably due to the formation of mineral plugs within the dentinal tubules thereby reducing the dissociation of hydroxyl ions and gradually tends to attain equilibrium by 28$^{th}$ day. In resorbed teeth, the change in pH is -0.6 on the 3rd day, -1 on 7th day, -1.2 on 14th day, -1.1 on 21st day and -0.8 on 28th day (Table 19). This may be attributed to the partial absence of cemental barrier which permits higher diffusion on day 3. Due to formation of mineral plugs within the dentinal tubules the diffusion of calcium and hydroxyl ions into the deionized water is reduced by 14th day and this gradually tries to attain equilibrium by the 28th day. The sudden fall in pH between 7th and 14th day could also be due to the super saturation of the deionized water. So, it can be concluded that the composition of the sealer (Sealapex) plays a role in the diffusion of hydroxyl ions both during primary endodontic treatment and retreatment.

MTA Fillapex (Group C) is a salicylate resin-based sealer containing tricalcium silicate particles, silicon dioxide and bismuth oxide. MTA Fillapex exhibits pseudoplastic behavior, (i.e) they decrease in viscosity when subjected to an increase in shear rate during compaction.[44] According to Rai et al. viscosity of MTA Fillapex increases from temperature 25°C to 37°C, thus improving the flowability and tubular penetration.[7] Silva et al (2015) showed that MTA Fillapex penetrated more deeply into the dentinal tubules than Sealapex and Pulp Canal Sealer EWT (zinc oxide eugenol based sealer). Another notable feature was that use of the intracanal calcium hydroxide dressings did not interfere with the penetration of MTA Fillapex sealer, due to the affinity between MTA and Calcium hydroxide residues.[2]

When comparing the pH change in intact teeth (Group C) during primary endodontic treatment and retreatment, there was a definite fall in pH on the 3rd day (- 1.6) decreasing by -1.1 on 7th day, -1.1 and 14th day, -1 on 21st day and -0.8 on the 28th day (Table 18). Initially the drop in pH may be attributed to the reason that MTA Fillapex is a calcium hydroxide releasing cement and also penetrates deep into the dentinal tubules, thereby initially reducing the pH by 3rd day, attaining maximum diffusion by day 7 and tending to attain equilibrium by 28th day. In resorbed teeth, the change in pH is -0.7 on the 3rd day, -1.2 on 7th day, -1.3 on 14th day, -1.1 on 21st day and -0.8 on 28th day (Table 19). This may be attributed to the partial absence of cemental barrier which permits higher diffusion on day 3 and also due the better penetrability of MTA even in the presence of left over calcium hydroxide. This marginally increases by day 7. The comparative reduction of pH from day 7 (pH-11.12) to 14 (pH 8.8) may be due to the supersaturation of the deionized water due to diffusion of calcium and hydroxyl ions, thereby reducing the pH (-2.3) and then gradually attains equilibrium by the 28th day. So, it can be concluded that the composition of the sealer (MTA fillapex) plays a role in the diffusion of hydroxyl ions both during primary endodontic treatment and retreatment. Further, this MTA based sealer shows better penetrability of calcium hydroxide which would initiate a mineralized layer formation even in the absence of cementum.

BioRoot RCS (Group D) is a hydraulic tricalcium silicate-based cement available as powder liquid system. The powder contains tricalcium silicate, povidone and zirconium oxide; the liquid is an aqueous solution of calcium chloride and polycarboxylate. Small particle size of this sealer permits deepest penetration into the dentinal tubules. BioRoot RCS is superior to MTA fillapex as it expands slightly (<0.1%) whereas MTA Fillapex shrinks (0.7%) during setting.[28] This shrinkage is due to the presence of salicylate resin in MTA fillapex sealer.[2] Bio-Root RCS exhibits mineral plugs in the dentinal tubules called **mineral infiltration zone**, this can be due to the manifestation of their biological activity.[51]

When comparing the pH change in intact teeth during primary endodontic treatment and retreatment in BioRoot RCS (Group D), there was a definite fall in pH on the 3rd day (- 1.8) decreasing by -1.1 on 7th day, -1.3 on 14th day, -1.4 on 21st day and -0.9 on the 28th day (Table 18). Initially the drop in pH may be attributed to the reason that Bio-Root RCS, having smaller particle size penetrates deeper into the dentinal tubules thereby initially reducing the pH by 3rd day, later on as BioRoot RCS is highly soluble it permits release of hydroxyl ions attaining maximum diffusion by day 7. The ability to form a **mineralization zone** within dentinal tubules causes a reduction in pH by day 14 and tends to attain equilibrium by 28th day. In resorbed teeth, the change in pH is -1 on the 3rd day, -1.3 on 7th day, -1.4 on 14th day, -1.2 on 21st day and -0.9 on 28th day (Table 19). This may be attributed to the partial absence of cemental barrier which permits higher diffusion on day 3. There is marginal increase in pH on day 7 due to its better penetrability. The comparative reduction in pH from day 7 to day 14 may be due to the **mineralization zone** formed by BioRoot RCS which is bioactive tricalcium silicate cement. Due to diffusion of calcium and hydroxyl ions the deionized water becomes supersaturated by 14$^{th}$ day and tries to attain equilibrium by the 28$^{th}$ day. So, it can be concluded that the composition of the sealer (BioRoot RCS) plays a role in the diffusion of hydroxyl ions both during primary endodontic treatment and retreatment. Since BioRoot RCS has the ability to form a **mineralization zone** within the dentinal tubules it may probably also form a similar mineralization zone in resorbed areas.

Thus, it is evidenced that the remaining calcium hydroxide from the initial root canal treatment, the depth of penetration of sealers and the rate of mineral plug formation in dentinal tubules are the main reasons for variations in the hydroxyl ion penetration through dentinal tubules during retreatment.

The overall hydroxyl ion diffusion during retreatment, irrespective of the sealers used, showed that in Group 1 (intact teeth) the pH of deionized water increased to approximately 10.02 on the 3$^{rd}$ day and reached a peak pH of approximately 11 on the 7$^{th}$ day. Then, the pH reduced gradually from 14$^{th}$ day (9.11) reaching a pH of approximately 8.66 on the 28$^{th}$ day. In Group 2 (resorbed teeth) pH raises faster for the 1$^{st}$ three days raising the pH approximately to 10.97. By the end of 1 week peak pH value of approximately 11.32 was obtained, which was marginally higher than the pH values obtained in intact teeth. By the end of day 14, the pH lowered to 8.9 and by day 28 reached a pH of approximately 8.53 (Table 20; Graph 8). Inter group comparison between Group 1 and Group 2 shows that there was significant difference in hydroxyl ion diffusion at all time periods, in both intact and resorbed teeth (Table 21).

In both the Groups (intact and resorbed teeth) there was a significant change in pH from 3rd to 28th days (Table 22). It is seen that diffusion of hydroxyl ions was more rapid during the first three days probably due to the use of aqueous based calcium hydroxide medicament. The peak diffusion rate was noted in the 7th day followed by gradual decrease on 14th,21st and 28th days.

This was similar to Safavi and Nakayama studies[37], who showed decrease in pH on the 14th day compared to 7th day perhaps due to the faster ion dissolution. The antimicrobial action of calcium hydroxide depends on the concentration of hydroxyl ions present in the solution. In slightly soluble substances, such as calcium hydroxide, provided that some undissolved solute is present in the saturated solution, the concentration of ions in the solution remains constant. This shows that even after hydroxyl ions are utilized on reaction to bacteria, the dissolution of calcium hydroxide will still continue to maintain the equilibrium. Thus, an aqueous preparation of calcium hydroxide can potentially maintain its high pH for a long time in the root canal.[48]

Sonali et al[42] showed that maximum pH of RC cal was noted at one week which gradually decreased with time as observed after one month. Buch et al[5] showed that while using RC Cal and calcium hydroxide points the pH raised gradually till 1 week followed by decline in pH at 14th day, whereas conventional calcium hydroxide powder mixed with distilled water showed raise in pH till $14^{th}$ day. On the contrary, Nerwich et al. 1993, Estrela et al. 1998, Felippe 1998, Hosoya et al. 2001 studies have shown that pH increased gradually and peaked after 2 weeks of $Ca(OH)_2$ placement. This is probably due to the form of calcium hydroxide medicament used. In all these studies, calcium hydroxide was used in powder form.

On comparison of pH changes during primary endodontic treatment and retreatment in intact teeth (Table 23; Graph 9) and also in resorbed teeth (Table 24; Graph 10), the mean pH at all time intervals showed that after placement of calcium hydroxide intracanal medicament, the amount of hydroxyl ion diffusion during primary endodontic treatment was significantly higher than the amount of diffusion during retreatment. Thus, it is shown that the remaining left-over calcium hydroxide and root canal sealers within the dentinal tubules have a significant role in lowering the diffusion of hydroxyl ions from calcium hydroxide intracanal medicament placed during retreatment.

Tao et al. 1991[46] showed that increased removal of root dentin during retreatment facilitated the passage of hydroxyl ions to the external root surface. Similarly, Roggendorf et al (2010)[35] showed that increased enlargement of the root canal reduces the amount of filling material in the dentinal tubules and thus improves retreatment efficacy.

Since the sealers caused changes in diffusion of hydroxyl ions from calcium hydroxide present in them, the pH change of calcium hydroxide was dependent on the type of sealer being used. Hence the hypothesis that the rate of hydroxyl ion diffusion from calcium hydroxide medicament is not dependent on the type of sealer being used in retreatment cases, stands rejected.

Enterococcus faecalis has been found to be the most common microorganism isolated in retreatment cases. The pH required for the inactivation of Enterococcus faecalis is around 11.[14] Christopher P et al (2004) proved that no growth of E. faecalis occurred from pH 11.5 to pH 12 and a pH of 10.5 to 11.0 retards E. faecalis growth.[6]

Thus, it can be suggested that in case of retreatment irrespective of the sealer used during initial endodontic therapy, the calcium hydroxide medicament placed during retreatment needs to be replaced at the end of 7 days to maintain the pH around 11 or teeth should be obturated within 4 to7 days of placement of the medicament.

Evaluating the efficacy of the sealers used in this study it is perceived that Sealapex was significantly less soluble than MTA Fillapex due to the presence of zinc stearate in Sealapex, which is known to be highly hydrophobic and thus prevented the ingress of water.[4] MTA Fillapex shrinks (0.7%) during setting forming gaps at the sealer/dentine interface which might lead to microbial invasion and reinfection. BioRoot RCS is thus considerably superior to MTA fillapex as it expands slightly (<0.1%) after setting.[28]

On considering the mineralizing ability it was seen that BioRoot RCS > MTA Fillapex > Sealapex, whereas zinc oxide eugenol did not have the ability to produce mineralization. Prolonged release of calcium ions by bioactivity of BioRoot RCS may promote endodontic and periodontal tissue regeneration.[39]

An ideal root canal sealer must not only perform a good and stable seal and prevent recurrent infections, but also be able to promote periapical tissue regeneration. Thus, within the limitations of the present study, it can be suggested that, Sealapex, MTA fillapex or BioRoot RCS can be used for obturation as it has an ability to penetrate deeply into the canals and its high alkalinity potential encourages both antimicrobial and mineralization action.

Contemplating between these 3 sealers it can be proposed that usage of BioRoot RCS is more advantageous, despite the fact that its retrievability is difficult, as it can maintain its alkalinity for about 30 days which is considerably higher than Sealapex and MTA fillapex. It is also suggested that in case of retreatment more amount of inner root dentin can be removed to facilitate increased amount of sealer removal and thereby accentuate penetration of hydroxyl ions from calcium hydroxide medicament.

In inflammatory root resorption, conventional treatment with long term calcium hydroxide dressings may cause lowering of fracture resistance. Hence it can be suggested that calcium hydroxide dressing for a week followed by obturation with GP and BioRoot RCS sealer may be considered, as it has shown to maintain alkaline pH for prolonged periods without weakening root dentin.

In the present study for simulating resorptive defects burs were used to create regular wells, whereas external inflammatory resorption results in irregular areas of denuded cementum. Additionally, this was an invitro study where a constant 10ml of deionized water was used, whereas clinically the quantity of periapical fluid is not constant and the continuous tissue fluid circulation may facilitate diffusion rather than equilibration. Consequently, the duration of time taken for supersaturation of external environment with calcium and hydroxyl ions might vary. Hence, further in vivo studies are needed to evaluate the rate of hydroxyl ion diffusion in intact and resorbed roots.

# *Summary*

## SUMMARY

The purpose of the present study was (i) to evaluate the hydroxyl ion diffusion from calcium hydroxide intracanal medicament in intact and resorbed teeth before and after retreatment and (ii) to assess if the hydroxyl ion diffusion from calcium hydroxide medicament during retreatment was dependent on the type of sealer used during primary endodontic treatment.

40 single rooted mandibular premolars were divided into 2 groups of 20 teeth each namely Group 1: Intact roots and Group 2: Simulated resorbed roots. All teeth were decoronated at CEJ followed by root canal preparation using Protaper Universal rotary instruments and copiously irrigated with 3% NaOCl and 17% EDTA and final rinse was done with saline. The canals were completely dried using paper points and intracanal dressing was done with RC Cal. The access cavity was closed with IRM and sealed with varnish. All the specimens were then suspended in a 10ml of deionized water and incubated at 37$^{o}$C. A digital pH meter was used to measure the pH on the 3$^{rd}$ ,7$^{th}$ ,14$^{th}$ ,21$^{st}$ and 28$^{th}$ day after placement of calcium hydroxide intracanal medicament.

Subsequently, the root samples were retrieved from their respective containers. Calcium hydroxide intracanal medicament was removed by copious irrigation and ultrasonic activation. The samples in Group 1 and Group 2 were further subdivided into 4 groups each based on the sealers used for obturation namely Group A (Zinc oxide eugenol sealer), Group B (Sealapex), Group C (MTA fillapex) and Group D (BioRoot RCS). The root canals were then dried

with paper points and the teeth in each subgroup were obturated with the respective root canal sealers and 2% gutta-percha points by lateral condensation technique. After 7 days the root fillings were then retrieved in all the samples using protaper universal retreatment files and copiously irrigated with 3% NaOCl and 17% EDTA. The removal of root filling material from the canal walls were verified using RVG.

The canals were then completely dried using paper points and intracanal medication of the canal walls was done with RC Cal. The access cavity was closed with IRM to prevent coronal leaching of calcium hydroxide. All the specimens were then suspended in a fresh solution of 10ml deionized water and incubated at 37$^{o}$C. A digital pH meter was used to measure the pH of the samples on 3$^{rd}$ ,7$^{th}$ ,14$^{th}$ ,21$^{st}$ and 28$^{th}$ day of the study.

Data obtained were compiled systematically in Microsoft Excel spreadsheet. Statistical analyses were performed in Statistical Package for Social Sciences software (SPSS IBM) using a personal computer and specific statistical tests (Kruskal-Wallis Test and Mann-Whitney Test) were used to find out the statistical significance of the obtained results.

# *Conclusion*

# CONCLUSION

The purpose of the present study was (i) to evaluate the hydroxyl ion diffusion from calcium hydroxide intracanal medicament placed in intact and resorbed teeth during primary endodontic treatment and retreatment and (ii) to assess if the hydroxyl ion diffusion from calcium hydroxide medicament during retreatment was dependent on the type of sealer used during primary endodontic treatment.

Within the limitations of this invitro study it can be concluded that:

1. The rate of hydroxyl ion diffusion measured by the **pH change** varied from a maximum of 12.04 to a minimum of 9.3 over the study period of 28 days **in intact teeth.**
2. The variation in **pH change in simulated resorbed teeth** was between 12.3 and 9.2 during the study period of 28 days.
3. The **pH changes** due to calcium hydroxide medicament **with time,** was shown as 7$^{th}$ day > 3$^{rd}$ day > 14$^{th}$ day > 21$^{st}$ day > 28$^{th}$ day in **intact teeth** during primary endodontic treatment.
4. The **pH changes** due to calcium hydroxide medicament **with time, in simulated resorbed roots** was similar to intact teeth during primary endodontic treatment showing 7$^{th}$ day > 3$^{rd}$ day > 14$^{th}$ day > 21$^{st}$ day > 28$^{th}$ day.

During primary endodontic treatment, the pH change in both intact and resorbed teeth was in the alkaline phase for the entire study period of 28 days. This is due to the dissociation of calcium and hydroxyl ions from the calcium hydroxide intracanal medicament diffusing through the dentinal tubules.

5. **The change in pH** was more alkaline in resorbed teeth than in intact teeth **during primary endodontic treatment** on **3$^{rd}$ and 7$^{th}$ day**, whereas, pH values on the **14$^{th}$,21$^{st}$ and 28$^{th}$ day** showed that the pH was higher in the intact teeth than resorbed teeth. This is due to the absence of the cemental barrier causing faster dissociation of calcium and hydroxyl ions in resorbed teeth for one week and then becoming supersaturated by the end of 2 weeks.
6. **During retreatment**, the rate of hydroxyl ion diffusion measured by the **pH change** varied from a maximum of 11 to a minimum of 8.6 over the study period of 28 days **in intact teeth**.
7. While **doing retreatment** the variation in **pH change in resorbed teeth** was between 11.3 and 8.5 during the study period of 28 days.
8. The **pH changes** due to calcium hydroxide medicament **with time,** was shown as 7$^{th}$ day > 3$^{rd}$ day > 14$^{th}$ day > 21$^{st}$ day > 28$^{th}$ day in **intact teeth** during retreatment.
9. The **pH changes** due to calcium hydroxide medicament **with time,** in **simulated resorbed teeth** was similar to intact teeth during retreatment showing 7$^{th}$ day > 3$^{rd}$ day > 14$^{th}$ day > 21$^{st}$ day > 28$^{th}$ day.

During retreatment, the pH change in both intact and resorbed teeth was in the alkaline phase for the entire study period of 28 days. This is due to the dissociation of calcium and hydroxyl ions from the calcium hydroxide intracanal medicament diffusing through the dentinal tubules.

10. **During retreatment**, the **change in pH** was more alkaline in resorbed teeth than in intact teeth on **3$^{rd}$ and 7$^{th}$ day,** whereas, pH values on the **14$^{th}$,21$^{st}$ and 28$^{th}$ day** showed that the pH was higher in the intact teeth than resorbed teeth. This is due to the absence of the cemental barrier causing faster dissociation of calcium and hydroxyl ions in resorbed teeth for one week and then becoming supersaturated by the end of 2 weeks.
11. The amount of hydroxyl ion diffusion (change in pH) **during primary endodontic treatment** was significantly higher than the amount of diffusion during **retreatment** at all time intervals.

    This may be due to the presence of remaining calcium hydroxide and sealer from the primary root canal treatment. Diffusion of hydroxyl ions from calcium hydroxide medicament during retreatment was found to be dependent on the composition and penetrability of the root canal sealers used in initial endodontic treatment (ZnOE sealer, Sealapex, MTA fillapex or BioRoot RCS).
12. **During retreatment** in both intact and resorbed teeth **after retrieval of GP and root canal sealer**, highest pH was observed in Group A (Zinc oxide

eugenol) followed by Group B (Sealapex), Group C (MTA fillapex) and Group D (BioRoot RCS) during the entire period of study.

Zinc oxide eugenol is not a hydroxyl ion dissociating sealer and therefore permits more diffusion of hydroxyl ions from calcium hydroxide intracanal medicament placed during retreatment. Sealapex showed marginally higher pH throughout the study in comparison to MTA fillapex and BioRoot RCS, as all these are hydroxyl ion dissociating sealers and the difference may probably be due the depth of penetration and solubility of these sealers.

**13. During retreatment**, after placement of calcium hydroxide medicament following removal of the sealer, in both intact and resorbed teeth, the peak value of pH was attained by end of **7 days** which gradually reduced by the **end of the study.**

14. Irrespective of the sealer retrieved, **in intact teeth** the peak **change in pH** occurred on the 7$^{th}$ day **during retreatment** followed by end of **3$^{rd}$ day**. The study showed that there was a fall in pH from **14$^{th}$ day to 28$^{th}$ day**. Thus, it can be suggested that in case of retreatment in intact teeth, irrespective of the sealer used during primary endodontic therapy, the calcium hydroxide medicament placed during retreatment needs to be replaced at the end of 7 days to maintain the pH around 11 or teeth should be obturated within 4 to 7 days of placement of the medicament to sustain the antimicrobial activity.

15. Irrespective of the sealer retrieved, **in resorbed teeth** the peak **change in pH** occurred by end of **7$^{th}$ day**, marginally increasing from the **3$^{rd}$ day**. The study showed that there was a significant fall in pH on the **14$^{th}$ day**, gradually decreasing towards **28$^{th}$ day**. So, in case of retreatment in resorbed teeth, irrespective of the sealer used during primary endodontic therapy, the calcium hydroxide medicament placed during retreatment needs to be replaced between 7$^{th}$ day and 14$^{th}$ day to maintain the pH above 9 as mineralization is likely to occur between pH 8.5 to 10.5.

On overall comparison of the results done invitro between intact and simulated resorbed teeth, and comparing primary endodontic treatment and retreatment it can be concluded that placement of calcium hydroxide medication helps in maintaining the alkalinity of the surrounding medium. This may enhance the antibacterial activity and the sealer used may also play a role on the diffusivity of hydroxyl ions which may contribute to mineralization. Hence, further studies need to be done on the long-term mineralizing ability and timing of obturation when using these newer root canal sealers, especially in resorbed teeth.

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# *Annexures*

# ANNEXURE –I

**RAGAS DENTAL COLLEGE & HOSPITAL**
(Unit of Ragas Educational Society)
Recognized by the Dental Council of India, New Delhi
Affiliated to The Tamilnadu Dr. M.G.R. Medical University, Chennai
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**TO WHOMSOEVER IT MAY CONCERN**

Date: 6.1.2018

Place: Chennai

From
The Institutional Review Board,
Ragas Dental College & Hospital,
Uthandi,
Chennai – 600119.

The dissertation topic titled **"EVALUATION OF HYDROXYL ION DIFFUSION FROM CALCIUM HYDROXIDE MEDICAMENT PLACED IN INTACT AND RESORBED TEETH DURING PRIMARY ENDODONTIC TREATMENT AND RETREATMENT – AN INVITRO STUDY"** submitted by **Dr. CELENA BENCY.M** has been approved by the Institutional Review Board of Ragas Dental College & Hospital.

**Dr. N.S. AZHAGARASAN, M.D.S.,**
Member Secretary,
Institutional Review Board,
Ragas Dental College & Hospital,
Uthandi,
Chennai – 600 119.

RAGAS DENTAL COLLEGE Chennai

# ANNEXURE –II

URKUND

## Urkund Analysis Result

Analysed Document: Bency thesis.pdf (D34287392)
Submitted: 1/2/2018 5:09:00 PM
Submitted By: drbenz15@gmail.com
Significance: 1 %

### Sources included in the report:

DISSERTATION FINNALE.docx (D34282702)
The thesis.docx (D29466640)

### Instances where selected sources appear:

2